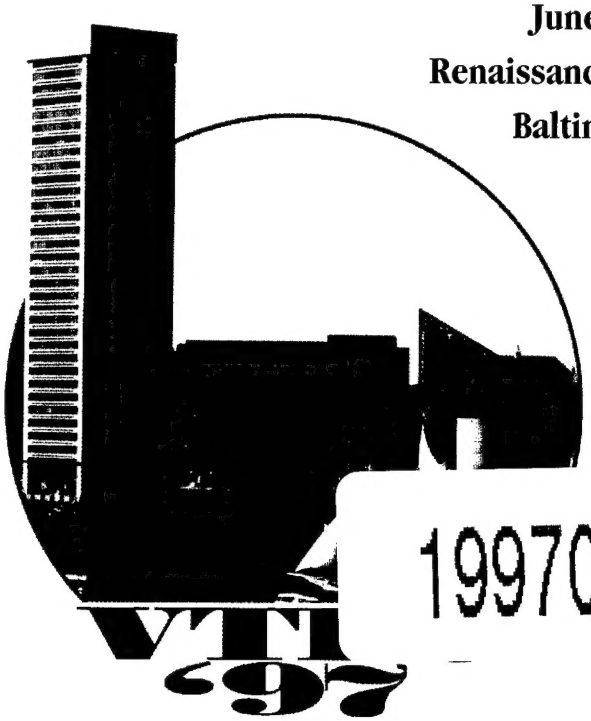
VTEC '97

3rd International Symposium
and Workshop on
Shiga Toxin (Verocytotoxin) – Producing *Escherichia Coli* Infections

June 22–26, 1997
Renaissance Harborplace Hotel
Baltimore, Maryland
USA



LOIS JOY GALLER FOUNDATION FOR HEMOLYTIC UREMIC SYNDROME, INC.

under the auspices of the Lois Joy Galler Foundation for Hemolytic Uremic Syndrome, Inc.

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Introduction

Dear Colleague,

It gives me great pleasure to welcome you to Baltimore and VTEC '97, the Third International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-producing Escherichia coli *infections.*

During the decade between VTEC '87, held in Toronto, and this 3rd symposium in Baltimore, there has been an explosive growth of knowledge about Shiga Toxin (Verocytotoxin)-producing Escherichia coli *(VTEC). At the same time there has been a worrisome increase in the incidence and impact of infections due to these organisms throughout the world. The major recent outbreaks in Japan, Germany and the western United States are cases in point. A lot of work remains to be done to control, manage and ultimately prevent the human suffering associated with this emerging infectious disease.*

The VTEC Symposium series was designed to provide a multidisciplinary forum for exchanging information, disseminating new knowledge, and highlighting state-of-the-art scientific advances in this rapidly evolving field. This core element of the Symposium remains intact in VTEC '97 as does the striving to achieve synthesis between art, science, humanity and good fellowship, a mission for the Symposium that became so firmly entrenched during the outstanding VTEC '94 meeting in Bergamo.

I wish to acknowledge the Co-chairmen of VTEC '97, Mr. Robert C. Galler without whose drive, dedication, and leadership, VTEC '97 would not have got off the ground, and Dr. James B. Kaper who has been committed to providing delegates with the best hospitality his home town of Baltimore can offer. In addition, Jim and Dr. Alison O'Brien have put together an outstanding Scientific Program.

As was the case at VTEC '87 and VTEC '94 we are pleased to see a wide array of disciplines represented at VTEC '97—pediatrics, nephrologists, hematologists, gastroenterologists, microbiologists, internists, veterinarians, food scientists, public health experts, biochemists, immunologists, molecular biologists, and many others.

Welcome to Baltimore! I hope you enjoy VTEC '97!

Mohamed A. Karmali
Chairman Emeritus, VTEC '97

Letters from the Chairmen

Dear Attendees,

Welcome to VTEC '97! The Lois Joy Galler Foundation for Hemolytic Uremic Syndrome, Inc. is proud to be part of the Third International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing Esherichia coli *Infections. VTEC '97 brings together experts from around the world that will gather to share and discuss topics and issues of utmost importance. The Scientific Program Committee, chaired by Drs. James B. Kaper and Alison O'Brien, has prepared an outstanding program for you!*

The Lois Joy Galler Foundation was established some four years ago in memory of my daughter, Lois Joy. The Foundation's goal were threefold—acquire funds for research to develop a cure and treatment for HUS, raise public awareness, and provide a supportive community for the families of affected children. I am proud to say that these goals have been met in many of the Foundation's accomplishments. We were instrumental in bringing about a grant from the National Institutes of Health in excess of $15 million dollars for research related to HUS. In addition, my wife Laurie and I were recently invited guests of President Clinton as he announced sweeping reform of the Federal food safety rules for meat and poultry. The new rules modernize a 90-year-old inspection program to reduce harmful bacteria.

I look forward to even greater strides in the near future and extend my sincere gratitude and appreciation for the contributions that will be made by all of you. Once again, welcome to Baltimore and my best wishes for a successful meeting.

Robert C. Galler
President, Lois Joy Galler Foundation
Co-Chair, VTEC '97

Dear Colleagues,

It gives me great pleasure to welcome you to Baltimore for the Third International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing Esherichia coli *Infections (VTEC '97). This meeting continues the strong scientific tradition established by VTEC '87 in Toronto and VTEC '94 in Bergamo.*

The scientific program planned for VTEC '97 will provide overview presentations and the newest contributions from a wide range of disciplines. The eight plenary sessions and two poster sessions are scheduled without concurrent sessions so that maximum "cross fertilization" among attendees with diverse backgrounds can occur. The plenary sessions will feature invited speakers who will present state-of-the-art overviews as well as short oral presentations selected from the submitted abstracts. I want to thank Alison O'Brien, Vice-Chair of the Scientific Program Committee, for her hard work in planning the scientific program and Mohamed Karmali and the other members of the Scientific Program Committee and International Advisory Board for their invaluable advice.

The scope of topics encompassed in the field of Shiga toxin (verocytotoxin)-producing E. coli *is astonishing, ranging from farm management of livestock to clinical management of end-stage renal disease. VTEC '97 will provide a multi-disciplinary meeting in which food scientists, veterinary scientists, and public health officials can talk to nephrologists, gastroenterologists, cell biologists, and infectious disease specialists. Such inter-disciplinary exchanges are critical for further advancements in understanding, treating, and preventing these infections.*

I am particularly pleased to be able to show you a bit of Baltimore, a revitalized city with a long history (long for the U.S.) of international trade, culture, and scientific achievement. The convenient location of all meeting facilities, hotel rooms, numerous restaurants, and a variety of tourist attractions within the attractive Baltimore Inner Harbor area will provide many opportunities for formal and informal interactions among the attendees. An extensive social program will also foster the renewal of old friendships and collaborations as well as the formation of new relationships.

I hope you will have a highly informative meeting, a very enjoyable stay in Baltimore, and great success in your research on this important topic.

James B. Kaper
Co-Chair, VTEC '97

General Information

Registration

The Registration Desk will be located on the fifth floor of the Renaissance Harborplace Hotel. Registration hours are as follows:

Sunday	June 22, 1997	12 noon-7pm
Monday	June 23, 1997	8am-6pm
Tuesday	June 24, 1997	8am-6pm
Wednesday	June 25, 1997	8am-5pm
Thursday	June 26, 1997	8am-12noon

Name Badges

Name badges will be distributed at the Registration Desk to all delegates. Delegates are kindly requested to wear the badge throughout the symposium. Only those wearing name badges will be admitted to the Scientific Sessions and Social Events.

To ensure safety, while visiting the areas outside of the hotel, please remove name badges.

Refreshments/Meals

Coffee will be served at approximately 10am and 3pm daily.

As lunch will not be provided by VTEC '97, delegates are encouraged to visit the Harborplace Gallery (downstairs from the Renaissance Hotel) and the shops and restaurants in the Inner Harbor (across the street from the Renaissance Hotel).

Poster Sessions

Formal Poster Sessions will be held in the Baltimore Ballroom of the Renaissance Harborplace Hotel. Although the following outlines the formal sessions, the Baltimore Ballroom will be open throughout the week—please be certain to visit at your leisure!

Monday	June 23, 1997	3:25pm-6:00pm
Tuesday	June 24, 1997	2:45pm-6:00pm

Social Program

Welcome Reception **Sunday, June 22** **7:30pm-11:00pm**

The Opening and Welcome Reception will begin at 7:30pm at the National Aquarium of Baltimore. The event will include cocktails, hors d'oeurves and a special, private viewing of the Aquarium.

An Evening at the George Peabody Library **Monday, June 23** **7:00pm-11:00pm**

A very special evening of music and dinner will be held at the George Peabody Library, renowned to be one of the most beautiful rooms in North America. Trolleys will depart the Renaissance Hotel Lobby starting at 6:45pm. Trolleys will shuttle delegates back and forth from the Library throughout the evening. The Library is located approximately 2 miles from the hotel.

Baltimore Crab Feast **Tuesday, June 24** **7:00pm-10:00pm**

Reservations have been made for VTEC '97 delegates at Obrycki's Crab House. Wear very casual clothes as you prepare to eat delicious crabs off newspaper covered tables with mallets and lots of beverage! Crabs are very spicy and eating them is an art in itself!

This is an OPTIONAL event and is not included in the registration fees. For those interested, please secure your reservation at the Registration Desk. The cost is $53.00 per person. Transportation will not be provided by VTEC '97, however, taxi cabs are available from the Renaissance Hotel Lobby.

Bay Lady Boat Cruise **Tuesday, June 24** **6:30pm-10:00pm**

All Aboard! The Bay Lady will set sail from the Inner Harbor for a three-hour cruise! Delegates will enjoy a delicious dinner buffet while slowly cruising through the beautiful Harbor. Boarding begins at 6:30pm, the ship set sails at 7:00pm.

This is an OPTIONAL event and is not included in the registration fees. For those interested, please secure your reservation at the Registration Desk. The cost is $37.00 per person. Transportation is not required, delegates can walk across to the Inner Harbor to board.

Closing Banquet **Wednesday, June 25** **7:00pm-1:00am**

The Closing Banquet will begin with cocktails at 7:00pm in the Maryland Foyer of the Renaissance Hotel. Dinner and Dancing will begin at 8:00pm. Delegates are encouraged to stay all evening for music, dancing and socializing.

The Organizing Committee of VTEC '97 graciously thank the following organizations and companies for their generous contributions.

Major Sponsors

Meridian Diagnostics, Inc.
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National Institute of Allergy and Infectious Diseases
National Institute of Diabetes, Digestive and Kidney Diseases

Monday, June 23

Final Program for VTEC '97

8:30–8:40 James B. Kaper, Robert C. Galler—Opening remarks

SESSION I
EPIDEMIOLOGY OF STEC INFECTIONS IN HUMANS (Chair: Michael Osterholm)

8:40–9:10 *Patricia Griffin*
Overview of epidemiology of STEC infections in humans

9:10–9:30 *John Spika*
STEC in Canada

9:30–9:50 *Hideshi Michino*
Investigation of outbreak of *E. coli* O157:H7 infection among school children in Sakai City, Japan, 1996

9:50–10:10 *Eduardo Lopez*
STEC in Latin America

10:10–10:30 *Alfredo Caprioli*
STEC Infections in Continental Europe

10:30–11:00 COFFEE

11:00–11:20 *Roy Robins-Browne*
Epidemiology of STEC in Australia

SUBMITTED TALK

11:20–11:35 *S. Ahmed, J.M. Cowden, M. Donaghy, W.J. Reilly, and A. Riley*
An outbreak of *E. coli* O157:H7 in Central Scotland

11:35–1:30 LUNCH—informal poster viewing

Monday, June 23

SESSION II
STEC IN THE FOOD CHAIN (Chair: Robert Tauxe)

1:30–1:55 *Dale Hancock*
Impact of farm management practices on incidence of STEC in animals

1:55–2:20 *Roger Johnson*
STEC in foods

2:20–2:45 *Jill Hollingsworth*
Food safety

2:45–3:10 *Dave Theno*
The response of the food industry to STEC

SUBMITTED TALK

3:10–3:25 *J. A. Shere, K. J. Bartlett and C. W. Kaspar*
Longitudinal study of *E. coli* O157 on four dairy farms in Wisconsin

3:25–6:00 POSTER SESSION (and COFFEE)

Tuesday, June 24

SESSION III
PATHOGENIC MECHANISMS: ADHERENCE AND TOXINS (Chair: Steve Calderwood)

8:30–8:55 *Phillip Tarr*
Intestinal adherence mechanisms

8:55–9:20 *Philip Sherman*
Epithelial cell signal transduction responses to STEC infection

9:20–9:45 *David Acheson*
Toxin delivery across an epithelial barrier

9:45–10:10 *Cliff Lingwood*
Toxin—receptor interactions

10:10–10:40 COFFEE

10:40–11:05 *Vernon Tesh*
Cytokine response to Shiga toxin

SUBMITTED TALKS

11:05–11:20 *P. D. Bloom, R. Russell, D. Blake, and E. Boedeker*
Interleukin-1 receptor antagonist (IL-1ra) protects against tissue injury in an animal model of hemorrhagic colitis

11:25–11:40 *G. Collington, I. Booth, M. Donnenberg, J. Kaper, and S. Knutton*
Attaching and effacing genes encoding secreted signalling proteins are also required for modulation of host cell electrolyte transport

11:40–1:15 LUNCH and informal poster viewing

Tuesday, June 24

SESSION IV
PATHOGENIC MECHANISMS: ANIMAl MODELS AND HOST RESPONSE
(Chair: Harley Moon)

1:15–1:40 *Evelyn Dean-Nystrom*
Bovine infections with *E. coli* O157:H7

1:40–2:05 *Brad Fenwick*
Canine model of HUS

2:05–2:30 *Mohamed Karmali*
Host immune response and immunity to VTEC/STEC infections

SUBMITTED TALK

2:30–2:45 *F.B. Taylor, Jr., L. DeBault, A.C.K. Chang, A. Li, V.L. Tesh, T.J. Pysher, R.L. Siegler*
Characterization of the primate (baboon) responses to Shigatoxin

2:45–6:00 POSTER SESSION (and COFFEE)

Wednesday, June 25

SESSION V
PATHOGENESIS OF HUS (Chair: Mark Taylor)

8:30–9:00 *Leo Monnens*
Pathophysiology of HUS

9:00–9:25 *Peter Rose*
Hematological aspects of HUS

9:25–9:50 *Caroline Savage*
Endothelial cell biology and participation of the endothelium in disease

9:50–10:20 COFFEE

10:20–10:45 *Tom Obrig*
Interaction of Shiga toxin with endothelial cells

SUBMITTED TALKS

10:45–11:00 *P.E. Ray, A. Onorio, J. Sgromo, M. S. Maglio, I. Marco, X-H. Liu, L. Xu, G. Gallo*
Increased release of basic fibroblast growth factor (bFGF) in children with classic hemolytic uremic syndrome

11:00–11:15 *J. Hutchison, D. Stanimirovic, A. Shapiro, G. Armstrong*
Verotoxin causes cytotoxicity in human cerebral endothelial cells

11:15–11:30 *R. Bhimma, N. Rollins, H. M. Coovadia, M. Adhakiri*
Hemolytic uremic syndrome following *Shigella dysenteriae* type 1 outbreak in South Africa

11:30–1:00 LUNCH and informal poster viewing

SESSION VI
DETECTION AND DIAGNOSIS OF STEC INFECTIONS (Chair: Wendy Johnson)

1:00–1:25 *Helge Karch*
Overview of detection methods

1:25–1:50 *Henry Smith*
Subtyping of VTEC

1:50–2:10 Discussion

Wednesday, June 25

SESSION VII
TREATMENT OF DISEASE DUE TO STEC (Chair: Bernard Kaplan)

2:10–2:35 *Marguerite A. Neill*
Infectious disease management

2:35–3:00 *Kevin Meyers*
Treatment of HUS and other complications

3:00–3:30 COFFEE

3:30–4:10 *Glen D. Armstrong and Peter Rowe*
Clinical trials of Synsorb Pk in preventing HUS

SUBMITTED TALKS

4:10–4:25 *A. Edwards, K. Arbuthnott, J.R. Stinson, H.C. Wong, C. Schmitt, and A. O'Brien*
Humanization of monoclonal antibodies against *Escherichia coli* toxins Stx1 and Stx2

4:25–4:40 *T. Takeda, M. Tanimura, K. Yoshino, E. Matsuda, H. Uchida, and N. Ikeda*
Early use of antibiotics for STEC O157 infection reduces the risk of hemolytic uremic syndrome

4:40–4:55 *A.I. Stewart, G.A. Jones, J. McMenamin, A.K.R. Chaudhuri, and W.T.A. Todd*
Central Scotland *Escherichia coli* O157 outbreak (Clinical Aspects)

4:55–5:45 Roundtable discussion
Bernard Kaplan
Marguerite A. Neill
Gianfranco Rizzoni
Mark Taylor
Richard Siegler
Phillip Tarr

Thursday, June 26

SESSION VIII
VACCINES AGAINST STEC (Chair: Myron M. Levine)

8:30–8:55	*Robert V. Tauxe* Public health immunization strategies: Who or what would we immunize?
8:55–9:20	*Gerald Keusch* Passive and active immunization against STEC and HUS
9:20–9:45	*Shousun C. Szu* LPs-based vaccines
9:45–10:10	*Carlton Gyles* Vaccines in animals
10:10–10:40	COFFEE

CLOSING SESSION
James Kaper and Alison O'Brien, Chairs

10:40–11:30	Formulation of questions for VTEC 2000 General discussion
11:30	Closing remarks—Robert Galler and James Kaper

TRANSMISSION OF VTEC O157 IN WELSH HOMES

V1/I

Sharon Parry*, Roland Salmon Public Health Laboratory Service, Communicable Disease Surveillance Centre (Welsh Unit), Cardiff, UK.

To estimate the extent of household transmission in Wales, over 2 years, all household contacts of microbiologically confirmed cases of VTEC O157 were identified, faecal specimens requested and their age, sex and diarrhoeal symptoms, from 7 days prior to the onset of the index case to date of interview, recorded. 83 cases had 181 household contacts. 101 (56%) submitted faecal specimens. 15 (8%) were excreting VTEC O157. 6/15 were asymptomatic, 7/15 had onsets after the index case, 2/15 had onsets before the index case, giving an estimated household transmission rate of (13/181) 7%. Non-hospitalised cases were more likely to transmit than hospitalised (12/102 vs 3/79, RR = 3.1, p = 0.05). Of 79 household contacts of index cases aged under 5, 12 (15%) were infected. Of 20 household contacts themselves aged under 5, 5 (25%) were infected. Transmission from under 5's to under 5's was 4/13 (31%). Surveillance in Wales is particularly complete. Household spread occurs most readily between children under 5. Investigation of sporadic VTEC O157 must include (i) advice on hygiene measures to prevent household spread (ii) identification of those contacts at risk of spreading the infection more widely.

ISOLATION OF SORBITOL-FERMENTING (SF) VEROCYTOTOXIN (VT)2-PRODUCING *E.COLI* O157:H- IN THE CZECH REPUBLIC

V9/I

M.Bielaszewska*, J.Janda, K.Bláhová, H.Karch, M.A.Karmali, M.A.Preston, R.Khakhria, O.Nyč
Ústav lék.mikrobiologie a Pediatrická klinika, 2.lék.fakulta University Karlovy, Praha, Česká republika; Institut für Hygiene und Mikrobiologie, Universität Würzburg, BRD; Dept.of Microbiology, The Hospital for Sick Children and Central Public Health Laboratory, Toronto, and Laboratory Centre for Disease Control, Ottawa, Canada

SF *E.coli* O157:H- strains are widespread in Germany but have not been reported from other countries. In 1995, 2 SF *E.coli* O157:H- strains were isolated in the Czech Republic from epidemiologically unrelated cases of hemolytic uremic syndrome. Both the strains fermented sorbitol within 24 hrs. and were β-glucuronidase-positive; they produced VT2 upon isolation but lost the VT during laboratory storage. They had atypical phage types and closely related PFGE patterns which differed from those of sorbitol-negative *E.coli* O157:H7 strains. One of the strains tested for the presence of *eae* and EHEC-hly genes harboured both of them. The characteristics of SF *E.coli* O157:H- strains isolated in the Czech Republic were close to those of such strains isolated in Germany suggesting that the strains might belong to the same clone.

V10/I

PRODUCTION OF VEROCYTOTOXIN (VT) 2 BY *ESCHERICHIA COLI* SEROGROUP O26

M.Bielaszewska*, H.Karch, J.Janda, K.Bláhová, O.Nyč
Ústav lékařské mikrobiologie a Pediatrická klinika, 2.lékařská fakulta University Karlovy, Praha, Česká republika; Institut für Hygiene und Mikrobiologie, Universität Würzburg, Würzburg, BRD

VT2-producing (VT2+) *E.coli* O26 strains have been rarely associated with human disease. The aim of this study was to establish the frequency of VT2 production in *E.coli* O26:H11/H- strains isolated from children with hemolytic uremic syndrome and diarrhea in the Czech Republic between 1963 and 1995 and further characterize the VT2+ isolates. Using the Vero cell neutralization test, VT2 phenotype was found in 2 of 101 strains isolated between 1963 and 1991 (namely, in 1966 and 1976), and in all 3 strains isolated in 1992-95. All the 5 VT2+ isolates harboured the VT2 gene and *E.coli* attaching and effacing (eaeA) gene as detected by PCR; 3 of them produced enterohemolysin and harboured the specific (EHEC-hly) gene. Two strains were sensitive to all 24 antibiotics tested, 3 strains were resistant to one or more of them. We conclude that VT2+ *E.coli* O26 strains were associated with human disease in the Czech Republic as early as 30 years ago and were rare till 1991;the frequency of their isolation markedly increased in 1992-95.

V11/I

E. COLI 0157 IN SOUTH LANARKSHIRE, SCOTLAND (1987-1996):-

THE CALM BEFORE THE STORM

Kenneth Liddell, Department of Microbiology, Law Hospital, Carluke, Lanarkshire Scotland.

Between November and December 1996, a major outbreak of *E. coli* 0157 infection (Phage Type 2, VT 2+) unfolded in Central Scotland. Its epicentre, in Wishaw, lies within the area served by Law Hospital. The outbreak was first identified and the majority of the primary isolations were made by this laboratory. Though Scotland has a high rate of infection with verocytotoxin-producing *E. coli*, Lanarkshire has, within a Scottish context, a comparatively low prevalence. Within the southern sector of the county, including Wishaw, this appears to be even lower. Data are presented on cases and isolates seen since 1987, when selective screening began, until the recent outbreak. Despite extension of screening, the incidence fell from 1991 to November 1996, and, in two single years, no isolations were made. An impression of low local prevalence should not undermine the need to remain vigilant in policing *E. coli* 0157. Laboratories should screen all diarrhoeal stools, without qualification.

SHIGA TOXIN - PRODUCING *ESCHERICHIA COLI* (STEC) IN GASTROINTESTINAL INFECTIONS IN FINLAND

V14/I

Marjut Saari, Markku Keskimäki, Ritvaleena Puohiniemi, Anja Siitonen*
Laboratory of Enteric Pathogens, National Public Health Institute, Helsinki, Finland

From February 1996 through January 1997 a total of 489 primary stool cultures from patients with bloody diarrhea were investigated by PCR for the presence of Shiga toxin-producing enteric bacteria. The cultures were received from 23 laboratories all over Finland. Eight cultures carried the genes encoding Shiga toxin production: seven were positive for stx_2 gene, one for stx_1. Of these, only four were positive for *eaeA*-gene. Pure cultures were obtained of seven and all of them were identified as *E. coli*. The strains positive for stx_2 belonged to four *E. coli* serotypes: OX3:H21, O157:H7, R:H49 and ONT:HNT. These strains produced Stx2 toxin as well; only O157:H7 strains were sorbitol negative. Two patients suffered from hemolytic uremic syndrome. In two cases, the finding was associated with a recent trip abroad.
This evaluation showed that diarrheal infections caused by STEC, including O157:H7, are rare in Finland.

ASSOCIATIONS BETWEEN HUMAN INFECTION WITH VERO CYTOTOXIN-PRODUCING *ESCHERICHIA COLI* O157 AND FARM ANIMAL CONTACT

V28/I

W.Barrie Trevena*, Geraldine A Willshaw, Tom Cheasty, Clifford Wray. Environmental Health Department, Kerrier District Council, Camborne, UK; Laboratory of Enteric Pathogens, Colindale, London, UK; and Central Veterinary Laboratory, Weybridge, UK.

Two main routes (ie. Food and Person-to-person) of transmission of Vero cytotoxin-producing *E. coli* O157 (VTEC O157) are well established and documented. This case-control study, now in its third year, explores the nature and extent of direct zoonotic transmission via contact with animals or their faeces. Cases are all laboratory confirmed isolates of VTEC O157 occurring within Cornwall and West Devon, with matched controls selected from General Practice registers. On-farm veterinary investigations are organised whenever a case has been in contact with farm animals. Matching of VTEC O157 strains isolated from human cases and from animals with which they have been in contact has been demonstrated on 9 occasions using a combination of phage typing and DNA methods, indicating an association between human illness and animal carriage (viz. cows, calves, a pony, a goat and a dog).

V33/I

HUMAN *ESCHERICHIA COLI* O157:H7 INFECTION ASSOCIATED WITH THE CONSUMPTION OF UNPASTEURIZED GOAT MILK

M.Bielaszewska*, J.Janda, K.Bláhová, H.Minaříková, E.Jílková, M.A.Karmali, J.Laubová, J.Šikulová, M.A.Preston, R.Khakhria, H.Karch, H.Klazarová, O.Nyč

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A cluster of *E.coli* O157 infection including hemolytic uremic syndrome, diarrhea, and asymptomatic cases occured in Northern Bohemia in 1995 following consumption of raw goat milk. Verocytotoxin 2-producing *E.coli* O157:H7 strains of phage type 2 and of identical pulsed-field gel electrophoresis patterns were isolated from the goat and from one of the patients. The frequency of anti-O157 lipopolysaccharide antibodies was significantly higher in the goat milk consumers than in control population (33% v. 0%;P=0.0005). These findings indicate that goats may be a reservoir of *E.coli* O157:H7 and a source of the infection for humans.

V37/I

ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN AUSTRIA

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In Austria no cases of EHEC O157 infections were diagnosed from 1991 till June 1992. From June 1992 till end of 1996, 5 out of 39 patients (12.8%) with culturally confirmed EHEC O157 infections, mostly from the western provinces Vorarlberg and Tyrol, developed HUS. O157:H7 and O157:H^- are the dominating sero-groups. Aside from transmission via contaminated food (the infections could not be traced to a particular source), direct transmission from person to person played a major role in the chain of these EHEC infections. In the Tyrol 3% of raw cow milk samples, 10% of ground meat samples, and 6% of calves yield EHEC O157. Despite this high rate of food-contamination, Austria—in contrast to neighboring countries like Italy and Germany—has not experienced a major outbreak with this organism so far. A nationwide surveillance system for HUS showed an incidence of 0.37 HUS-cases per 100.000 residents in the age group 0–14 years for 1995.

EPEC REVISITED: MORE THAN HALF OF THE DANISH ENTEROPATHOGENIC *E. COLI* (EPEC) STRAINS OF O GROUPS O26, O111 AND O128 ISOLATED 1959-1996 ARE ACTUALLY VTEC

V41/I

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The International *Escherichia* and *Klebsiella* Centre (WHO), Department of Gastrointestinal Infections, Statens Serum Institut, Copenhagen, Denmark

A random collection of 142 *E. coli* strains isolated from faeces in the years 1959-1996 in Denmark and belonging to EPEC O groups O26, O111 and O128 were examined for VT production and with DNA probes VT1, VT2, *eae*A, EAF and EHEC (pCVD419). 81 (57%) were VTEC. The earliest VTEC strain, serotype O26:H11, was isolated in 1959. 69/102 O26:[H11], 11/29 O111 strains and 1/11 O128 strains were VTEC. O26 was the most common and uniform group with 62 strains reacting with VT1, *eae*A and EHEC probes. Only 3 O26 strains were $VT2^+$ whereas 65 VTEC and 14 EPEC strains were $EHEC^+$. 58% (69 VT^+ and 14 VT^- strains) were $EHEC^+$. Only 4% were EAF^+. The study shows that more than half of EPEC O groups O26, O111 and O128 isolated in Denmark are actually VTEC and that 85% of these are $EHEC^+$. These results raise questions about the true etiology of diarrhoea in Denmark and confirm that $EHEC^+$ strains are quite common and that EAF^+ strains are rare in Europe.

EPEC REVISITED: SOME OF THE DANISH ENTEROPATHOGENIC *E. COLI* (EPEC) STRAINS OF O GROUPS O26, O111 AND O128 ISOLATED 1959-1996 ARE ACTUALLY VTEC

V42/I

Fleming Scheutz*, The International *Escherichia* and *Klebsiella* Centre (WHO), Department of Gastrointestinal Infections, Statens Serum Institut, Copenhagen, Denmark

A collection of 142 *E. coli* strains isolated from faeces in the years 1959-1996 in Denmark and belonging to EPEC O groups O26, O111 and O128 were examined for VT production and with DNA probes VT1, VT2, *eae*A, EAF and EHEC (pCVD419), 81 (57%) were VTEC. The earliest VTEC strain, serotype O26:H11, was isolated in 1959, 69/102 O26:[H11], 11/29 O111 strains and 1/11 O128 strains were VTEC, O26 was the most uniform group with 62 strains reacting with VT1, *eae*A and EHEC probes. Only 3 O26 strains were $VT2^+$ whereas 65 VTEC and 14 EPEC strains were $EHEC^+$, 58% (69 VT^+ and 14 VT strains) were $EHEC^+$. Only 4% were EAF^+. The study shows that some of EPEC O groups O26, O111 and O128 isolated in Denmark are actually VTEC and that many of these are $EHEC^+$. These results raise questions about the true etiology of diarrhoea in Denmark and confirm that $EHEC^+$ strains are quite common and that EAF^+ strains are rare in Europe.

V44/1 SEROTYPES OF SHIGA-LIKE TOXIN-PRODUCING *ESCHERICHIA COLI* (SLTEC)ISOLATED FROM HUMAN AND ENVIRONMENTAL SOURCES IN AUSTRALIA.

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Shiga-like Toxin-producing *Escherichia coli* (SLTEC) have been isolated from both human infections and environmental sources in Australia for many years. In many cases they have been submitted to this laboratory for full serotyping. Although strains belonging to serotype O157:H7 have been identified, these have been in the minority. A number of other serotypes most notably O5:H-; O26:H11; O48:H21; O91:H-; O111:H-, O113:H21 and O128:H2 have also been identified from human cases. While some of these have also been found in environmental sources including domestic animals and meat, these have revealed an even greater variety of serotypes, many of which have as yet not been isolated from human cases here or elsewhere according to reports in the literature. Apart from O and H serotyping, employing a full range of antisera, the strains were also tested for their ability to produce SLT by both an immunoassay as well as by their reactions in Vero-cells. They were also tested for their ability to ferment sorbitol, their haemolytic characteristics as well as their reactions on the two new media CHROMagar O157 and Rainbow agar O157. The importance of these non-O157 SLTEC will be assessed, and possible methods for their identification considered.

V46/1 LOOKING BEYOND ENTEROHAEMORRHAGIC *ESCHERICHIA COLI* O157:H7

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For 15 years Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 tended to dominate the world literature on EHEC. They are probably derived from one particularly successful clone which has spread globally, colonizing domestic animals and causing human disease. For many more years, there have been reports of human cases and outbreaks of disease due to non-O157:H7 EHEC. These may occur concommitantly with O157:H7 cases. Such outbreaks may be falsely labelled as due to this serotype. Evidence will be presented from the literature and from investigations of one outbreak, that the ease of identification of the O157:H7 clone, can confuse the issue. While not denigrating the role of the O157:H7 clone, this paper illustrates the importance of recognizing that other serotypes can also be responsible for outbreaks as well as sporadic human disease.

ESCHERICHIA COLI ON THE INFORMATION SUPERHIGHWAY. V47/I

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The *E. coli* Index was established on the WWW in March 1995. Since then a number of subsections have been added. The one on pathogenic *E. coli* is in brief summary form with up-to-date references. During outbreaks of VTEC such as the recent one in Scotland the page was accessed by at least 5,000 people. This constitutes an important means of providing correct and current information to the public. The apparently increasing numbers of such outbreaks throughout the world demands dissemination of reliable information, for which the WWW is an ideal vehicle. The readership distribution of these pages will be presented, together with a discussion of possible future directions in which information about pathogens, and VTEC in particular can be effectively presented on the WWW.

A NATIONWIDE ASSESSMENT OF DIAGNOSTIC FACILITIES FOR *ESCHERICHIA COLI* O157 INFECTIONS IN ITALY V50/I

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We carried out a nationwide assessment of diagnostic facilities for the diagnosis of *E. coli* O157 infection in humans. Standardized mail questionnaires were sent to public microbiology laboratories in the whole country. Data collection and analysis is at present still in progress, however preliminary results are available for 10 out of 20 regions of Italy. These preliminary results indicate that most laboratories use sorbitol McConkey (83%) and/or latex agglutination assay (92%) as diagnostic tools. Sixty-two percent seek *E. coli* O157 in feces when asked by the clinicians, 37% in all bloody diarrhea samples, 33% in all diarrhea samples, and 8% in all feces submitted to the laboratories. A proportion ranging from 0 to 1% of all samples screened in the last 5 years in each laboratory yielded positive results for *E. coli* O157. Since most laboratories seek *E. coli* O157 on request made by the patient's physician the real burden of *E. coli* O157 infection is probably underestimated in Italy.

V52/I

EPIDEMIOLOGY OF SHIGA-TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) INFECTION IN CONTINENTAL EUROPE

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In most countries of continental Europe STEC infection has been recognized as an important public health problem later than in North America and UK, and appears to exhibit a rather different epidemiologic pattern. To date, human infections by STEC O157 have been described in 16 countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, The Netherlands, Norway, Poland, Slovenia, Spain, Sweden, and Switzerland), while specific surveys failed to isolate this organism in Malta and Croatia. Infections with non-O157 STEC serogroups were frequently reported: O111 in 9 countries, O26 in 8, 0103 in 7, O128 in 5. According to studies performed in 9 countries, STEC infection was detected in 0.4% (Denmark) to 2.7% (Germany) of patients with diarrhea. Four countries reported outbreaks of infection by STEC O157, and 3 by STEC O111. Most of these outbreaks occurred in the community at large and their source was not identified. The diffuse circulation of non-O157 STEC and the occurrence of communitywide outbreaks not associated with an evident source of infection appear to be the hallmarks of STEC infection in continental Europe.

V55/I

SURVEILLANCE OF HEMOLYTIC UREMIC SYNDROME (HUS) IN ITALY: 1988-1996

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The nationwide surveillance system of HUS was introduced in Italy in 1988. Up to December 1996, 175 cases were notified, accounting for a mean annual incidence of 0.2×10^{-5} in the age group 0-15. The mean age of patients was 40 months, 70% of them had prodromal diarrhea, 42% bloody diarrhea, and 66% required dialysis. Neurologic symptoms were observed in 37% of cases and 7% had coma. The notification trend remained constant over time, except in 1992 and 1993 when 2 clusters of cases were observed. Evidence of infection with Shiga-toxin (Stx)-producing *E.coli* (STEC) was shown in 73% of patients. STEC and fecal Stx were detected in 8% and 27% of cases, respectively. Stx-neutralizing antibodies and antibodies to the lipopolysaccharide of serogroups O157, O111, O26, and O103 were found in 10% and 59% of cases, respectively. The STEC serogroup most commonly associated to HUS was by far O157, followed by O111, O26, and O103.

MOLECULAR EPIDEMIOLOGY OF VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* ISOLATES IN JAPAN 1996 USING PULSED-FIELD GEL ELECTROPHORESIS

V68/I

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Pulsed-field gel electrophoresis (PFGE) using restriction enzyme *Xba*I was applied for molecular typing of 1,706 verocytotoxin-producing *Escherichia coli* (VTEC) O157 isolates, which were derived from 19 outbreaks, sporadic cases, foods, beef fecal swabs and environments in Japan 1996. One hundred eighty VTEC O157 isolates (53 from seven outbreaks in May-June, 222 from sporadic cases, three from foods and two from fecal swabs) showed very closely related patterns. Only one of the three food isolates, however, was epidemiologically correlated to an outbreak. Three hundred twenty five VTEC O157 isolates (95 from other eight outbreaks in July, 227 from sporadic cases, one from food and two from environments) showed different PFGE patterns from those of the May-June outbreak isolates. The food isolate of these cases is not correlated to any outbreaks. In addition, various VTEC O157 strains were isolated from the other outbreaks and sporadic cases in Japan 1996. Various types of VTEC O157 strains have already spread over Japan.

A MULTISTATE OUTBREAK OF *ESCHERICHIA COLI* O157:H7 INFECTIONS ASSOCIATED WITH EATING MESCLUN MIX LETTUCE

V74/I

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Between May 21 and June 21, 1996, 28 isolates of *Escherichia coli* O157:H7 with indistinguishable patterns on pulsed-field gel electrophoresis (PFGE) examination were received by the Illinois Department of Public Health. A study involving age-, sex-, and telephone-exchange-matched patients and controls revealed an association between *E. coli* O157:H7 infection and consumption of mesclun mix lettuce, a mixture of different types of baby lettuces (matched odds ratio: undefined, 95% confidence interval: 1.4-infinity, p=0.009). During the same time, the Connecticut Department of Health also implicated mesclun mix in an outbreak of *E. coli* O157:H7 infections. Isolates from the outbreaks in Illinois and Connecticut were indistinguishable by PFGE. Lettuce traceback revealed one grower as the likely source of lettuce implicated in both outbreaks; cattle were found next to the lettuce growing and processing areas. This is the fifth lettuce-associated outbreak of *E. coli* O157:H7 infections in North America. Current lettuce production practices should be evaluated for microbiological safety.

V75/I A REVIEW OF OUTBREAKS OF VERO CYTOTOXIN PRODUCING *ESCHERICHIA COLI* O157 IN ENGLAND AND WALES, 1992-1996

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In 1992 an enhanced surveillance system was introduced to collect epidemiological data on general outbreaks of Vero cytotoxin producing *Escherichia coli* O157 (VTEC O157) infection in England and Wales. Between January 1992 and December 1996, 37 outbreaks were investigated in which 381 people were affected, 59 developed HUS, 120 were admitted to hospital and 14 died. The route of transmission was foodborne in 27 outbreaks. The foods implicated included: cold cooked meat; milk; raw vegetables; cooked ground beef dishes. There were five outbreaks where person to person spread predominated, three occurred in pre-school nurseries, one in a hospital and one in a residential home for the elderly. In two outbreaks illness was acquired through direct contact with farm animals. The route of transmission remained unidentified in three outbreaks. Detailed updated information on the epidemiology of these outbreaks will be presented. Figures for 1996 are provisional.

V84/I PINPOINTING HUMAN INFECTION OF *E.coli* 0157 FROM ANIMALS

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The routes of transmission of *E.coli* 0157 infection between animals and humans, whether direct or indirect, have not been clearly defined. Previously, possession of an identical phage type for both animal and human isolates was deemed sufficient as a direct infective link. Since 1994, 24% of all *E.coli* 0157 isolates received by the Scottish Reference Laboratory have been isolated from animals including cattle, sheep, goats, geese and a horse. Conventional and genotypic methods (AP-PCR, PFGE, DNA sequencing) were applied to define this group accurately. Sixteen incidents were identified where both the human case and the implicated animal(s) shared the same phage type. Thirteen incidents were caused by phage type 2 or 28 isolates, the most common phage types in humans (75% of infections in Scotland in 1996). In eleven of the sixteen incidents the human and animal isolates shared identical PFGE restriction fragment profiles. These isolates were also indistinguishable by the molecular methods. Investigation of these incidents has demonstrated that genetically indistinguishable sub-types of *E.coli* 0157 exist in both animal and human populations.

IDENTIFICATION OF A RECURRENT CLONE ASSOCIATED WITH OUTBREAKS OF *E.COLI* 0157 INFECTION IN SCOTLAND

V86/I

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Scotland has one of the highest incidences of *E.coli* 0157 infection in the world; even within the U.K. the observed incidence is several times greater than that for England, Wales or N. Ireland. Systematic analyses of disparate isolates of *E.coli* 0157 have demonstrated that those of a common phage type (pt 2) can be sub-divided into 10 distinct pulsed field groups. A recurrent clone has been identified with a particular macrorestriction profile from multiple veterinary food, environmental and clinical sources throughout Scotland from 1989 to the present day. The consistent pulsed field gel electrphoretic profiles obtained for multiple isolates of the recurrent clone are indistinguishable following cleavage with six different endonucleases. In contrast to other pt2 strains, it has been responsible for several major outbreaks of infection including the largest milk-borne outbreak in the world and the recent outbreak in which 18 people died. The physiological reasons for prevalence of this clone are as yet undetermined, but its identification and persistence in the Scottish 0157 population is significant.

GENETIC HETEROGENEITY OF *E.COLI* 0157:H7 AND ITS UTILITY IN STRAIN TYPING

V87/I

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In 1994 seven major outbreaks of *E.coli* 0157:H7 infection occurred throughout Scotland including the largest outbreak of milk-borne *E.coli* 0157:H7 infection to date world-wide. A variety of suspect vehicles of infection were identified and there were 144 confirmed cases in total. All isolates associated with the outbreaks were subjected to detailed sub-typing: phage typing VT-PCR and pulsed-field gel electrophoresis (PFGE). The outbreak strains were of three different phage types (2, 4 and 28), all VT-/VT2+ except for phage type 4 (VT1+/VT2+). In efforts to discriminate outbreak-associated isolates from the high sporadic background (4.73 cases/100,000 population) in 1994, real-time PFGE analyses were performed which demonstrated that within each of the seven outbreak groups the macrorestriction profiles observed were indistinguishable, whereas profiles for sporadic isolates were not. The consistent genetic heterogeneity observed within the Scottish *E.coli* 0157 population can be exploited in epidemiological investigations.

V88/I

MOLECULAR EPIDEMIOLOGY OF SCOTTISH *E.coli* O157 ISOLATES - A THREE YEAR STUDY OF PHAGE TYPE 28

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During the years 1994 - 1996, Scotland had a higher incidence of *E.coli* O157 infection than the rest of the U.K. exacting significant demands on health resources. Accurate high resolution typing of these organisms is essential so that their source and routes of transmission may be identified. During the three year study period, the Scottish Reference Laboratory for *E. coli* O157 received 880 isolates of which 30 % were phage type 28, one of the most common Scottish types. Following pulsed-field gel eletrophoresis (PFGE) of these isolates, 25 macrorestriction profile groups were generated allowing definitive characterisation to a level not previously possible This enabled the discrimination among isolates associated with different outbreaks, and also between outbreak and sporadic isolates. Interestingly, the most common profiles from clinical isolates were also the most common in cattle, sheep and goats. Although some profiles had been observed throughout the study period, eleven were only observed within the final six months reflecting the heterogeneous nature of the *E.coli* O157 population in Scotland at this time.

V90/I

TEMPORAL AND SPATIAL DISTRIBUTIONS OF HUMAN CASES OF VEROCYTOTOXIGENIC *ESCHERICHIA COLI* INFECTION IN SOUTHERN ONTARIO

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The temporal and spatial distributions of cases of verocytotoxigenic *Escherichia coli* (VTEC) infection were described using data on human cases reported for the southern region of Ontario, Canada, between 1990 and 1995. A temporal model of VTEC incidence was constructed which permitted the detection of clusters of cases in time. Counties with the highest incidence of human VTEC infection were situated in areas of predominantly mixed agriculture. There was a significant positive association between the geographical distribution of cattle density and human VTEC incidence ($p = 0.012$). These findings suggest an increased risk of infection by VTEC organisms for people living in rural areas as compared to urban centres. The importance of contact with cattle and the consumption of potentially contaminated well water and/or locally produced food products as risk factors for VTEC infection may have been previously underestimated.

AN OUTBREAK OF INFECTION DUE TO VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* O157 IN FOUR FAMILIES: THE INFLUENCE OF LABORATORY METHODS ON THE INVESTIGATION

V99/I

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A case of *E.coli* O157 infection was diagnosed in early November but it was several weeks later that a probable link between this case and illness in three other families was discovered. Faecal samples from eight cases and from six asymptomatic contacts were examined for *E.coli* O157 by culture onto cefixime tellurite sorbitol MacConkey agar, immunomagnetic separation (IMS) and for *E.coli* O157-specific secretory IgA by an enzyme immunoassay. One case was positive by direct culture and three were positive by IMS. Six were positive for specific IgA. Two cases were negative for IgA and excreted the organism for 60 and 89 days respectively. All asymptomatic family contacts were negative for both *E.coli* O157 and IgA. The study illustrates the value of sensitive methods in following up of cases of infection.

DURATION OF OLIGURIA AND ANURIA PREDICTS CHRONIC RENAL DAMAGE IN POST-DIARRHEAL (D+) HEMOLYTIC UREMIC SYNDROME (HUS)

V113/I

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We determined if the presence and duration of oliguria (olig) and anuria (anur) during the acute phase of D+ HUS predicted chronic renal damage one or more years later; 206 children were studied a median of 5 years post HUS (range 1-23 yrs). The incidence of chronic renal sequelae (at the most recent evaluation) in those with and without various periods of oliguria and anuria was as follows:

Duration	Proteinuria		Low GFR		Prot. or Low GFR		Prot. & Low GFR	
(days)	Olig	Anur	Olig	Anur	Olig	Anur	Olig	Anur
0	20%	17%	25%	26%	32%	37%	7%	5%
1-5	20%	23%	27%	26%	38%	41%	5%	8%
6-10	12%	29%	8%	25%	20%	50%	---	4%
>10	50%	71%	29%	57%	60%	86%	19%	43%

Approximately one-third of the non-oligoanuric patients have renal sequelae (proteinuria or low GFR); it appears to be severe (i.e., combined proteinuria and low GFR) in about 5%. The incidence did not increase significantly until the oligoanuria exceeded 10 days ($p = <.05$), and was most notable in those with prolonged anuria.

V116/I

RISK OF HEMOLYTIC UREMIC SYNDROME (HUS) FOLLOWING SPORADIC *E.COLI* 0157 INFECTION: RESULTS OF A CANADIAN COLLABORATIVE STUDY

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The objective of this study was to better estimate the age-specific risks of HUS and hemolytic anemia (HA) following *E.coli* 0157:H7 infection among a representative cohort of children from the Province of Alberta and to compare this to the rates in children presenting to tertiary care centres in the rest of Canada. Children with HUS or 0157:H7 gastroenteritis were eligible if they were less than 15 years of age, and a stool sample had been submitted to one of 18 participating labs or to one of two Provincial labs in Alberta. Children with 0157:H7 gastroenteritis had blood and urine samples at day 8-10 of the illness to ascertain for hemolysis, anemia, thrombocytopenia, and renal injury. From June 1991 to March 1994, HUS was diagnosed in 205 children. Of these, 77% had evidence of 0157:H7 infection. A further 586 children had 0157:H7 gastroenteritis, of whom 18 had HA. The risk of HUS following 0157:H7 infection in Alberta was 8.1% (95% CI, 5.3-11.6) compared to 31.1% in the rest of Canada. In Alberta, the highest age-specific risk of HUS/ HA was 13% in those less than 5 years of age. These data will help guide clinical care and provide a basis for estimating the sample sizes needed in future treatment trials for the secondary prevention of HUS.

V117/I

EFFECTS OF THE HEMOLYTIC UREMIC SYNDROME ON COGNITIVE, ACADEMIC AND BEHAVIOURAL FUNCTIONING

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While the occurrence of severe neurologic sequelae has been well documented following HUS, little information is available on the prevalence of clinically important abnormalities in cognitive function, academic performance, and behaviour in those who escape obvious and severe neurologic deficits during the acute illness. The objective of this study was to examine whether mild cognitive and behavioural abnormalities occur with greater frequency in HUS survivors than among controls. Ninety-one HUS survivors without obvious CNS sequelae at discharge were compared with hospital controls pair-matched on age, sex, socio-economic status, first language and history of acute hospital admission. Cognitive, academic and behavioral tests were administered by psychometrists blinded to patient-control status. No differences were obtained on cognitive nor academic measures [e.g., HUS vs controls: Full scale IQ, 104.9 (SD 13.6) vs 106.2 (12.9), p = .45; WIAT math, 101.7 (11.9) vs 99.8 (15.4), p = .40]. HUS survivors did not show any deficits on behavioural ratings. Children who make an uncomplicated recovery from an acute HUS episode are not at heightened risk for mild CNS sequelae.

CHARACTERIZATION OF *ESCHERICHIA COLI* 0157:H7 BY PHAGETYPING

V124/I

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Verocytotoxigenic *Escherichia coli* O157:H7 and other serotypes of VTEC have been reported as emerging food-borne pathogens in many countries. In Canada, among human isolates of VTEC, serotype O157:H7 has increased from 25 laboratory confirmed cases in 1982 to 1277 in 1995. Sporadic cases and outbreaks of O157:H7 have been reported from all provinces in Canada. Among the various phenotypic markers, phagetyping of this serotype has provided excellent strain discrimination in epidemiological investigations. During 1990-94, a total of 2425 human isolates, representing 1937 sporadic cases - 488 from 128 outbreaks and 33 cases from nonhuman sources were investigated by the phagetyping scheme for O157:H7 as described by Khakhria et al., (Epid. Infect, 1990, 105: 511: 520). In addition, VT gene typing by PCR of randomly selected 1994 O157:H7 isolates produced different VT genotypes: VT1, VT2, 80.9%; VT1, 1.3%, VT2, 8.4% and 9.0% of the isolates included a VT2v genotype. Phagetyping of E. coli O157:H7 has shown excellent discriminatory capability and can be used in conjunction with other markers to determine the epidemiological relationships between human and nonhuman isolates.

LATE GASTROINTESTINAL AND RENAL SEQUELAE FOLLOWING AN OUTBREAK OF E. COLI O157:H7-ASSOCIATED HUS IN WASHINGTON STATE. JR Brandt, MW Joseph, PI Tarr, SL Watkins. Children's Hospital and Medical Center, Seattle, WA.

V127/I

Twenty-nine survivors of an outbreak of E. coli O157:H7-associated hemolytic uremic syndrome (HUS) were studied prospectively to assess for and identify predictors of, adverse outcomes. At 3 years post-HUS none of the patients had a GFR < 90ml/min/1.73m2 nor an elevated serum creatinine. Four percent (1/29) had hypertension. Thirty-one percent (9/29) had developed nonnephrotic proteinuria or hematuria . A large number of patients (21%) suffered gastrointestinal sequelae including cholelithiasis requiring cholectomy (3/29), persistent pancreatitis (2/29), late colon stricture (1/29) and/or glucose intolerance requiring insulin therapy (1/29). Logistic regression analysis found gastrointestinal sequele was associated with hypertension or acute gastrointestinal complications during HUS. Thrombocytopenia lasting more than 10 days during HUS was associated with an increased risk of persistent urinary abnormalities (hematuria and proteinuria). These data suggest that children with E. coli associated HUS have evidence of good renal functional outcome 3 years post-HUS although minor urinary abnormalities may be seen. However, gastrointestinal sequelae can be significant in these children.

V128/I SURVEILLANCE OF *E.COLI* 0157 IN SCOTLAND

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Scotland has one of the highest rates of infection with *E.coli 0157* in the world. While most cases are sporadic, in recent years a large milkborne outbreak affected more than 100 people and a general outbreak, associated with a butcher's shop, involved over 410 patients of whom 18 died. The rate of infection in Scotland is several times greater than in other parts of the United Kingdom. Surveillance of laboratory confirmed infections demonstrates the consistent variation that occurs in different geographical areas within Scotland, ranging from 1.1/100,000 of the population to 17.3/100.000 in 1995, a year when only one outbreak was reported. In 1996 rates rose as high as 32.3/100.000. No explanation has been identified for this geographical variation. Reference laboratory typing, particularly phage typing has allowed the emergence of different strains, such as phage type 28, unknown before 1993, to be identified. This strain predominated in 1996 in both humans and animals, yet is relatively rare in the rest of the United Kingdom. This may give some clue for the high rate of infection in Scotland.

V132/I INCIDENCE OF HUS AND ROLE OF O157 AND NON-O157 VTEC INFECTION IN HUS IN BELGIUM

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To evaluate the incidence of HUS in Belgium and to determine the role of O157 and non-O157 VTEC, 22 centers registered all cases of HUS and when possible collected faecal samples for culture & PCR and serum for LPS antibodies (serotypes O157, O26, O91, O103 & O111). Forty-six cases of HUS (including 5 incomplete cases) were recorded in 36 children (32 post-diarrheic) and 10 adults (5 post-diarrheic). Stools or serum were available from 38 cases. Evidence of VTEC infection was found in 22 children and 1 adult: O157 in 16 cases, O157 + O26, O26, O111, O121, O172, O not typable in 1 case each; in one case no isolate was recovered in spite of a positive PCR for VT2. The yearly incidence of complete HUS was at least 4.2 cases/ 100 000 children < 5 year and 0.4 cases/100 000 inhabitants, comparable to other data from Europe and North America. More than one fourth of the cases were due to non-O157 VTEC, showing that other serotypes also play a role in HUS in Belgium.

ESCHERICHIA COLI O157 H7 AND URINARY TRACT INFECTION (UTI) V134/I

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Although the importance of *E. Coli* O157 H7 as a gastrointestinal pathogen is well recognized, there are a paucity of data regarding its role in extraintestinal sepsis. To investigate the hypothesis that this bacterium may be uropathogenic we examined 240 mid-stream specimens of urine from which a "coliform" had been isolated. Only specimens satisfying the Kass criteria, with a leucocyte count of > 50/cu.mm and an absence of epithelial cells on microscopy were examined. Putative *E. coli* isolates were examined by standard biochemical methods, assessment of colonial characteristics on cefixime-tellurite-sorbitol MacConkey and methyl umbelliferyl glucuronide agars using a multipoint-inoculation technique and slide agglutination with anti-O157 antiserum. Of 215 isolates confirmed as *E. coli*, none were identified as belonging to serotype O157 and conclude that this bacterium is not a common etiologic agent of uncomplicated UTI. A similar study with strains associated with pyelonephritis is underway. We are also currently examining strains of *E. coli* O157 from clinical, veterinary and food sources for expression of type 1 and P fimbrial adhesins which are the major virulence determinants of uropathogenic *E. coli* isolates to determine whether the rarity of O157 strains in urinary specimens is due to the absence of these factors.

A CASE-CONTROL STUDY OF SPORADIC CASES OF O157 AND NON-O157 VTEC INFECTION V136/I

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The aetiology of sporadic VTEC infection was investigated by means of a case-control study. Forty cases (8 O157, 13 *eae* + and 19 *eae* - non-O157 VTEC) and 74 matched controls were interviewed about contact with a person with diarrhoea or with animals, recent travel, outdoor activities, eating in a restaurant or in a fast-food establishment, consumption of untreated water and of various foods including raw or undercooked beef. In a preliminary matched analysis of the data using Epi-Info, 3 factors were found to be associated with an increased risk of becoming infected: consumption of fish ($P<0.00005$) and of fromage blanc ($P=0.01$) and contact with a person with diarrhoea ($P=0.048$). Person-to-person transmission and dairy products have already been reported as infection sources. Fish has not yet been associated with VTEC, although VTEC have been isolated from fresh seafood. Future studies should address the role of non-bovine foods.

V147/I INTERNATIONAL OUTBREAK OF *ESCHERICHIA COLI* O157:H7 INFECTIONS ASSOCIATED WITH UNPASTEURIZED COMMERCIAL APPLE JUICE

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In October 1996, 13 *Escherichia coli* O157:H7 (O157) isolates were identified in the Seattle area which were indistinguishable by an experimental, rapid DNA restriction fragment length polymorphism (RFLP) technique. In a case-control study, all 10 initial cases (defined as cases with a common RFLP pattern) but none of 9 acquaintance controls consumed Brand A unpasteurized apple juice (odds ratio=undefined, p=0.00001). Overall, four states and one Canadian province reported 71 cases (defined as O157 infection in a person who drank Brand A apple juice or had contact with a case-patient in the 10 days before illness began); 40 (56%) were in persons ≤5 years old (range 1 to 46 years). Twenty-five (36%) patients were hospitalized; 14 (20%) developed HUS; one died. A container of recalled apple juice grew O157 with RFLP and pulsed-field gel electrophoresis patterns indistinguishable from that of case isolates. In this outbreak, timely identification of a common strain and epidemiologic evidence prompted rapid public health action. Quality control practices at the Brand A state-of-the-art facility were insufficient to produce safe juice without the addition of a barrier to microbial growth, such as pasteurization.

V156/I DIFFERENCES IN *ESCHERICHIA COLI* O157:H7 ANNUAL INCIDENCE AMONG FOODNET ACTIVE SURVEILLANCE SITES

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To determine the magnitude of *Escherichia coli* O157:H7 infections in five sites across the US we initiated active laboratory-based surveillance and surveyed laboratories, physicians and the general public regarding factors associated with the diagnosis of *E. coli* O157:H7. In 1996, the combined annual incidence rate was 2.9/ 100,000 population, but varied widely by site [0.6 (GA), 1.1 (CA), 2.1 (CT), 2.6 (OR), 5.4 (MN)]. Only the laboratory practice of culturing all bloody stools for *E. coli* O157:H7 affected reporting by site. In CT, OR, and MN >90% of stools were cultured in laboratories that followed this practice, compared to <70% in CA and GA. Adjusting for physician and laboratory culture practices, incidence rates ranged from 1.4 (GA) to 7.8 (MN) cases/ 100,000. Differences in *E. coli* O157:H7 incidence rates by site were not explained by these factors. This suggests the risk of exposure to *E. coli* O157:H7 may vary by site.

HEMOLYTIC UREMIC SYNDROME CAUSED BY *E. COLI* 079:H7 V158/I

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Most patients with diarrhea-associated (D+) HUS are infected by Shiga toxin-producing *E. coli* 0157:H7. We describe a patient with D+ HUS infected with *E. coli* 079:H7, a serotype not previously documented to cause HUS in the USA. The patient is a previously healthy 15-year-old white male who presented with abdominal pain and bloody diarrhea followed by progressive thrombocytopenia, anemia and acute renal failure. He was anuric for 3 days and was treated with peritoneal dialysis for 9 days. He also received fresh frozen plasma (1 unit/day) for 4 days. Stool was negative for *E. coli* 0157:H7 in the hospital laboratory but CDC studies revealed *E. coli* 079:H7 which was shown to possess the stx_2 gene. A greater than four-fold rise in IgG antibody titer to *E. coli* 079 LPS was demonstrated by the ELISA method (acute titer =1:80; convalescent 1:1280) but serum antibodies to 0157:H7 were absent. The patient's GFR returned to normal (91 ml/min/1.73 m^2) only 3 weeks after he was started on dialysis. This case report underscores the fact that *E. coli* serotypes other than 0157:H7 may cause HUS in the USA.

TRENDS IN ANTIMICROBIOTIC RESISTANCE OF ENTEROHAE-MORRHAGIC *ESCHERICHIA COLI* O157 ISOLATED IN JAPAN V177/I

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A total of 311 enterohemorrhagic *Escherichia coli*(EHEC)O157strains from sporadic cases and outbreaks between 1984 and 1996 in Japan were studied for antimicrobial susceptibility using 9 antimicrobial agents ; chloramphenicol(CP), tetracycline(TC), streptomycin(SM), kanamycin(KM), ampicilin(ABPC), sulfamethoxazole– trimethoprim (ST), nalidixic acid(NA), fosfomycin(FOM) and norfloxacin(NFLX). In sporadic cases, 43(16.6%)of 259 strains were resistant.The resistance patterns were as follows ; TC(2.7%), SM(1.9%), ABPC(0.4%), TC·SM (4.6%),TC·ABPC(0.8%), SM·ABPC(1.9%), TC·SM·ABPC(3.5%), SM·ST ·ABPC(0.4%), CP·TC·SM·KM·ABPC(0.4%). The number of multiple resistant strains has been increasing since1993.On the otherhand, all of 52 strains from 9 outbreaks were susceptible to those antibiotics.

V178/I **A 13–YEAR STUDY OF ENTEROHAEMORRHAGIC *ESCHERICHIA COLI*(EHEC) INFECTIONS IN TOKYO (1984–1996)**

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In 1996, many outbreaks and sporadic cases unordinary have been reported throughout the country in Japan. In Tokyo, we had 2 outbreaks and 114 sporadic cases including 104 of serotype O157 and 10 of other serotypes. A total of 5 outbreaks have been documented in Tokyo between 1984 and 1995. Those outbreaks occured in young people such as in a primary school(3 outbreaks) and a nursery school (2outbreaks). On the other hand, 2 outbreaks in 1996 had occured at a barbecue restaurant and catering lunch box delicatessen. The barbecue restaurant outbreak was very interesting because initial 3 sporadic cases were found in quite different areas, and the epidemiological investigation, bacteriological and molecular biological analyses indicated the outbreak should be occurred at a restaurant.

V179/I **MOLECULAR EPIDEMIOLOGY OF VEROCYTOTOXIN–PRODUCING *ESCHERICHIA COLI*O157:H7 STRAINS ISOLATED IN OSAKA CITY, JAPAN, IN 1996**

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In 1996, we experienced a marked increase of patients suffering from enteritis due to Verocytotoxin–producing *Escherichia coli* O157:H7. There were 186 cases in 1996 but none was reported in 1995. To elucidate the reason why the number of patients had increased so dramatically, one hundred sixty–nine isolates were analyzed by pulsed–field gel electrophoresis (PFGE), phage typing (PT), and random amplified polymorphic DNA analysis (RAPD). Isolates were assigned to eleven different phage types, 33 different PFGE patterns, and five RAPD patterns. This study revealed that numbers of sporadic cases caused by *E. coli*O157 that occurred in 1996 had comprised a regional outbreak due to the organisms belonging to phage type 32. One hundred eleven isolates (65.7%) belonged to the type, and the dates of onset showed a peak on July 15. Some PT–32 strains were distinguished from the prevalent type by PFGE. PFGE was apparently more sensitive than the other methods for differentiation of strains; however, isolates showing the same PFGE pattern were divided into several different groups by PT. Thus by combining PFGE and PT, isolates were classified into 37 different groups, and thirty–nine different groups were identified by combining PFGE, PT, and RAPD. Phage typing provided rapid useful information to understand the occurrence of a regional outbreak, although it is not as discriminatory as PFGE. The use of both PT and PFGE enhances surveillance of *E. coli*O157.

FREQUENCY OF SHIGA-LIKE TOXIN-PRODUCING *ESCHERICHIA COLI* IN BRASILIA CHILDREN, DF, BRAZIL

V180/I

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The aim of this study was to determine the frequency with which Shiga-like toxin producing *Escherichia coli* are present in stools from Brasilia children, submitted for bacteriological analysis. A total of 675 *E. coli* strains isolated from 145 children with diarrhea and 143 *E. coli* strains isolated from 32 children without symptom of diarrhea were analysed for the presence of SLT1, SLT 2, eae and EAF genes, besides EHEC plasmids and hemolysin production. Hybridization with SLT 1 was found in 3 (0,4%) of 675 *E. coli* strains isolated from children with diarrhea. Two of those were positive for eae and EAF specific DNA sequences. None of the STEC strains produced hemolysin and all of them were negative for EHEC plasmids. All strains (either isolated from children with diarrhea or isolated from assymptomatic children) were negative for SLT 2. We found a low frequency of SLT producing strains among *E. coli* isolates. The relationship of SLT producing *E. coli* and EPEC strains associated with diarrhea are still unclear. Further investigations are necessary to characterize the "unconventional" STEC which were detected in our study and their possible role in human pathogenicity.

HEMOLYTIC UREMIC SYNDROME AND VEROTOXIN-PRODUCING *ESCHERICHIA COLI* INFECTION IN FRANCE

V184/I

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To determine the pediatric incidence of hemolytic uremic syndrome (HUS) in France and to specify the role of verotoxin-producing *Escherichia coli* (VTEC) infection, we conducted a study in collaboration with the French Society of Pediatric Nephrology using a retrospective review of all cases of HUS from January 1993 to March 1995 and a one year prospective study of clinical, epidemiological and microbiological features of HUS. 269 cases were reported between January 1993 and March 1996. The average incidence /year was $0.72/10^5$ children <15, $1.78/10^5$ children <5. PCR procedure has been used to detect VT, eae, ehly genes directly from stool samples. Sera samples were examined for antibodies to lipopolysaccaride (LPS) of 25 major VTEC serogroups. During the prospective study, 122/130 cases were examined for evidence of VTEC infection using PCR and/or serological assays. 105 (86%) had evidence of VTEC infection. VT genes were detected in stool samples in 58% of cases, antibodies to LPS O157 in 67%. This study showed that VTEC infection is an important cause of HUS in France, with a high prevalence of O157 serogroup.

V187/I FEATURES OF *SHIGELLA*-ASSOCIATED HEMOLYTIC UREMIC SYNDROME (HUS) IN CHILDREN.

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We determined the incidence and clinical features of HUS in 784 children aged <141 months with culture-proven shigellosis. Of the 20 (2.6%) children who developed HUS 14 (70%) were infected with *S. dysenteriae* type 1 (Sd1) and only 142 of 764 (18.6%) who did not develop HUS were infected by this serotype of *Shigella*. Significantly higher proportion of patients who developed HUS had a history of anorexia (100% vs. 71%; p = 0.002), abdominal pain (80% vs. 52%; p = 0.015) and tenderness (25% vs. 9%; p = 0.031, straining at stools (95% vs. 70%; p = 0.016), history of taking an antibiotic prior to hospitalization (45% vs. 20%; p = 0.011), features of ileus (20% vs. 4%; p = 0.007), and were better nourished (p = 0.018). Association of HUS with prior use of antibiotic and abnormal abdominal radiological findings persisted even when the analysis was restricted to children infected with Sd1. We conclude that HUS may also occur in association with serotypes other than Sd1, and that there is an association of HUS with use of antibiotics prior to hospitalization.

V191/I **AN OUTBREAK OF E.COLI 0157 IN CENTRAL SCOTLAND**

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On Friday 22 November 1996, Lanarkshire Health Board became aware of 15 possible cases of E.coli 0157 infection (5 of which had been confirmed micro-biologically). Initial investigation suggested that all but one of the cases had either consumed cold cooked meat products from a local butcher, or had eaten steak pie at a lunch on 17 November 1996 supplied by the same butcher. Immediate actions were taken to remove the suspected products from the food chain.

So far 415 cases have been identified with symptoms compatible with E.coli 0157 of whom 292 have been confirmed. The organism has been typed as phage type 2, VT1 negative, VT2 positive. E.coli 0157 of the same phage type have been isolated from the food and environmental samples taken from the butcher and all the isolates from these samples are indistinguishable from human cases on pulsed field gel electrophoresis (PFGE).

Sadly, 18 elderly patients have died making this one of the worst food poisoning outbreaks in this country. In total 151 cases have been admitted to hospital.

SUBTYPING OF ENTEROHEMORRHAGIC ESCHERICHIA COLI (EHEC) ISOLATED IN GERMANY, 1987 - 1996

V194/I

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1070 shiga-toxins (Stx)-producing strains were isolated and examined in Germany of which about 55% were recovered from human, 33% from animal and 11% from food. To determine the presence of Stx, cell-culture-tests, DNA-colony-blot-hybridization and the polymerase-chain-reaction were performed. A complete virulence pattern was examined in 354 strains (formation of Stx and EHEC haemolysin, presence of the *eae*A genes). Among the 239 identified serotypes, the predominant serotypes in human strains (311 strains) were E. coli O157:H7 and O157:H-. Within 203 examined E. coli serotypes of O157:H7/H-, 15 phage types could be differentiated. The four most common were 1,3,4 and 8. Based on the Sorbitol-fermentation and the ß-Glucuronidase-production, strains of the O157-group were divided into biovar 1 and 2 and subdivided into further 15 biovars by means of 4 biochemical reactions. Furthermore, data on the resistence of enterohemorrhagic O157 to antibiotical agents, isolated in Germany will be presented.

PHAGE TYPES OF *ESCHERICHIA COLI* O157 ISOLATES FROM HUMAN AND NON-HUMAN SOURCES ENGLAND AND WALES 1992-1996

V197/I

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During 1992-1996, 4115 isolates of Vero cytotoxin-producing *Escherichia coli* O157 were phage typed. There were 2718 isolates from humans, 1298 from animals and 99 from human foods. The animal isolates were from cattle, sheep, goats, pigs, a horse and seagulls. The predominant phage types (PTs) were 2, 49, 1, 4, 8, 21. Since our previous survey (1989-1991) we have observed changes amongst the predominant PTs associated with human infection both in relation to the proportion of strains belonging to individual PTs and also in the PTs most commonly associated with outbreaks. PT2 is still predominant, whereas the numbers of PT49 strains has fallen steadily since 1991. In 1994, PT 2 and PT49 accounted for 46% and 17% respectively of all human isolates, in 1996 PT2 strains totalled 37% and PT49 strains 6%. PTs 8 and 21 have increased significantly in numbers since 1994, becoming the second and third most common PTs found in England and Wales. Phage typing is a valuable tool enabling the rapid identification of outbreaks and epidemiological trends, and all *E. coli* O157 VTEC should be phage typed and VT subtyped. When the clinical or epidemiological situation warrants additional discrimination, further subtying using molecular-based techniques e.g. PFGE or RFLP analysis may then be initiated.

V208/I

HAEMOLYTIC-UREMIC SYNDROME FOLLOWING A *SHIGELLA DYSENTERIAE* TYPE 1 OUTBREAK IN SOUTH AFRICA

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An epidemic of *Shigella dysenteriae* type 1 (SD1) has changed the epidemiology of haemolytic-uraemic syndrome (HUS) in Southern Africa. In this region HUS was endemic but restricted to mainly white children in the northern provinces of South Africa and in Zimbabwe. We report on 81 cases of HUS occurring from July, 1994 to February, 1996, following an outbreak of SD1 dysentery in Kwazulu/Natal province of South Africa. All patients, excluding 1 child, were black (a group previously thought to be at low risk of HUS), with a mean age of 38 m; 50 were males. The mean duration of dysentery on admission was 11.3 days (range 1-41). The majority of patients had acute oliguric renal failure (90.1%); 42 required peritoneal dialysis. Stool culture for SD1 was positive in only 7 patients at the time of admission. Outcome was as follows: recovery 32; impaired renal function 8; chronic renal failure 26; end stage renal disease 1 and death 14 patients. Risk factors, the spectrum and severity of extra-renal complications, management and outcome of these patients differed from patients with *Escherichia coli*-associated HUS. The high mortality and morbidity in SD1-associated HUS presages the need for the development of novel therapy in the management of these patients, which can be used in developing counties.

V209/I

SEROLOGIC EVIDENCE OF INFECTION OF DAIRY FARM FAMILIES WITH NON-O157 VEROTOXIN-PRODUCING *ESCHERICHIA COLI*

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In a previous study, dairy farm family members (DFFM) more frequently had antibodies (Ab) to verotoxin (VT) 1 (41%) than to O157 lipopoly-saccharide (LPS) (12.5%). To determine if the higher rate of seroconversion to VT was associated with exposure to non-O157 VT-producing *Escherichia coli* (VTEC) harboured by cattle, we are testing sera from the 236 DFFM and 484 urban residents for Ab to LPS of 10 common bovine non-O157 VTEC serotypes. Results to date for serogroups O26, O111, O113, and O145 indicate a higher frequency of O111 LPS Ab (19%) and O145 LPS Ab (14%) than O157 LPS Ab in DFFM, and that Ab to LPS of O111, O113 and O145 serogroups are more frequently elevated in DFFM (8-19%) than in urban residents ($\leq$5%) ($p<0.05$). These preliminary findings provide further evidence for an elevated risk of VTEC infection in DFFM, and for the ability of non-O157 VTEC to infect humans.

RECURRENT DIARRHEA-ASSOCIATED HEMOLYTIC UREMIC SYNDROME (D+ HUS). V213/I

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The low frequency of antibodies to verotoxin-1 (VT-1) in patients with VT-1 producing Verotoxin-producing *E. coli* (VTEC) infection coupled with reports of 2 cases of recurrent D+ HUS or hemorrhagic colitis (HC) suggests that the development of protective immunity following a primary VTEC infection is limited. We report 3 cases (2 Canadian and 1 from King County, WA) of recurrent D+ HUS to strengthen the concept that primary VTEC infection, like tetanus, does not evoke protective immunity. Each patient (2 females and 1 male) was <2 years (yr) old when they developed either HC or D+ HUS. The etiology for each primary illness was not established although it is presumed to be VTEC infection. All 3 patients developed *E. coli* 0157:H7 associated HUS 2 yr., 4.5 yr. and 8.5 yr. respectively, following the original illness. In Canada, about 100 cases of HUS occur annually, and the risk of D+ HUS is estimated to be 1.4 cases/100,000 children <15 yr/annum. During the past 40 yr, at least 3 cases of recurrent HUS per estimated 4,000 primary HUS cases have been identified. This is substantially greater than the expected frequency of 1.4 cases per 100,000 primary HUS cases in the absence of any protective immunity. The reasons for these differences remain to be elucidated. The two stages of VTEC infection at which antibodies may be protective are colonization and toxemia. The apparent lack of protective immunity following a primary VTEC infection indicates that antibody responses following primary infection may be either inadequate or non protective with respect both to the toxins as well as to the colonization factors such as intimin and the secreted proteins.

TWO MAJOR CLONES WERE FOUND DURING A CRISIS OF VTEC O157:H7 (VT1+, VT2+) INFECTION IN JAPAN, 1996 V216/I

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Since the first outbreak of VTEC O157:H7 ($VT1^+$, $VT2^+$) in the end of May, we had more than 16 outbreaks in parallel with many sporadic cases in 1996. Two major peaks were seen in June and July. The highest peak involved 49 sporadic cases in a day in the middle of July. At the same time, the biggest outbreak in Sakai city was also reported. We carried out molecular analysis to know whether the strains were clonal or not. Many sporadic and outbreak strains from several areas in the middle of June were very similar in PFGE-pattern. The strains collected in July were different from June-isolates, though the July-isolates were very similar each other. Bovine strains from a central Japan were found to be the same in the PFGE-pattern to that of strains isolated in June.

V223/I PREVALENCE OF EHEC INFECTION IN CHILDREN WITH HUS AND HOUSEHOLD CONTACTS VERSUS MATCHED CONTROLS

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EHEC is associated with esporadic cases and outbreaks of bloody diarrhea and HUS in children. The source of infection is not clear as the main reservoir for EHEC are contaminated undercooked meat and meat products normally consumed by adults rather than children. We believe that asyntomathic adults may transmit EHEC to children through domestic contact. Using a case/control design, we studied 26 children with HUS and their families for EHEC infections. Also 75 children matched for age, socioeconomic level, and area of residence, along with their families were included as controls. Fecal samples were analyzed for EHEC by DNA probes and cytotoxin by ELISA (EHEC Premier, Meridian OH) considering infection positivity of either probes or cytotoxin. Isolates were tested for 26 different serogroups. EHEC infection was detected in 18 (69.2%) HUS cases and 19 (73.1%) of their families, versus 38 (50.6%) of control children ($p=0.1$), and 54 (72%) of their families (NS). Serogroup O157 was significantly more frequent in HUS cases and their household contacts than in controls. 15 of 37 strains isolated from cases versus 6 out 74 strains isolated for controls ($p=0.0001$). This case/control study gives further evidence that infection by EHEC serogroup O157 represents a risk factor for developing HUS.

V225/I EPIDEMIOLOGY OF VEROTOXIN-PRODUCING *ESCHERICHIA COLI* (VTEC) INFECTIONS IN ARGENTINA

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It has been estimated about 250-300 HUS cases per year occur in Buenos Aires. Because of this, we sought to define the role of Verotoxins in patients with HUS, bloody diarrhea (BD), watery diarrhea (WD) and healthy children (HC). Cattle have been described as the reservoir for bacteria that cause these diseases. In Argentina the bovine meat consumption per capita is extremely high. We therefore decided to study cattle and meat as potential reservoirs of VTEC. We studied: children with HUS $n=103$ (16 ± 9.6m), BD $n=254$ (18.8 ± 12.5m), WD $n=100$ (14.6 ± 8.7m) and HC $n=103$ (20.9 ± 11.1m). The incidence of SLT-associated (SLT-a) illness in Argentina was: WD: 21/100 (21%); HUS: 42/73 (57/5%) ($p<0.001$) and 99/254 (38.9%) of BD ($p<0.005$). *E.coli* O157:H7 was isolated in 3% of children. Our data show that 31.5% of cows had VTEC in their feces and 22.7% of the meat samples contained VTEC. CONCLUSIONS: 1) The high incidence of SLT-a BD presumably explains the unusual frequency of HUS in Argentina; 2) There is a significant difference between the incidence of SLT-a BD (39%) and SLT-a WD (21%)($p=0.002$); 3) Evidence of SLT-producing *E.coli* in fecal samples of cattle could explain the role of the animals as source of EHEC in Argentina.

A SURVEY OF THE PREVALENCE OF O157 VTEC IN RAW MEATS IN SOUTH-EAST SCOTLAND

V2/II

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Infections caused by verocytotoxigenic *E.coli* O157 (O157 VTEC) have emerged as a major public health concern in North America and in Europe. South-east Scotland has rates of O157 VTEC infection amongst the highest in the UK. Although meat products have been implicated in a number of outbreaks, the lack of suitable methods of sufficient sensitivity for the routine detection of the organism in foodstuffs has frustrated attempts to further define these associations. The recently developed technique of immunomagnetic separation (IMS) has revolutionised our ability to isolate the organism, with an increase in sensitivity of between ten and one-hundredfold. The current study aims to determine the proportion of retail meat samples in which O157 VTEC is present. 1000 samples of raw retail meats (80%beef; 20% lamb) submitted by local environmental health departments will be examined for O157 VTEC by the IMS technique. Results of the analysis of the first 300 specimens will be presented.

BEHAVIOUR OF *E. COLI* O157:H7 IN DRY FERMENTED SAUSAGES

V7/II

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The behaviour of *E. coli* O157:H7 in dry fermented sausages was studied in order to meet the demands of the American Food Safety Inspection Service (FSIS) and the German Federal Meat Association. Three different types of products namely a long fermented salami, a fat reduced salami and a "Teewurst" were produced and the batter inoculated with bacterial loads of 10^2 and 10^6 CFU/g. Different pools of *E. coli* O157:H7 isolates originating from stool, faeces and food samples were used as inocula. A reduction of the *E. coli* counts towards zero was noted in sausages after 4 and 12 weeks in long-time fermented salamis challenged with 10^2 and 10^6 CFU/g, respectively. In the lean type salami a reduction of one log in the samples inoculated with 10^2 *E. coli* was noted in the 5 th week, while a decrease of three log was observed in case of the higher inoculum. In contrast to these types of sausages similar degrees in reduction of *E. coli* could not be obtained in the Teewurst samples. This study demonstrates that long ripening of dry fermented sausages could enable reduction rates up to 5 $\log_{10}$ of *E. coli* O157:H7. It also could be proved that *E. coli* O157:H7 in dry fermented sausages is mainly dependent on the substratum (i.e. the composition of the batter), the technology of production used and moreover on the bacterial load itself and the combination of the isolates used as pool for the inoculation.

V13/II TRANSFORMATION OF SHIGATOXIN - ENCODING GENES

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We are studying if transformation of Shigatoxin-encoding genes can play a role in the spreading of the toxin genes. Free DNA can be released to the environment after heat-killing the bacterium. We have shown that even after autoclaving an overnight broth of Shigatoxinogenic *E. coli* O157:H7, specific stx_1 and stx_2 fragments can be amplified by PCR. Such autoclaved material was used in transformation experiments with XL-1 competent cells. Semi-nested PCR amplification of transformants with stx_1 and stx_2 - specific primer sets revealed PCR fragments about 200 basepairs longer than the products found when performing a similar PCR on *E. coli* O157:H7. Sequence analysis of these fragments will be presented. In light of the recent finding that *E. coli* is shown able to develop natural competence in freshwater, transformation experiments under conditions simulating natural environments are currently being performed.

V22/II A SURVEY OF RAW BEEF AND RAW MILK FROM NORTHERN IRELAND FOR THE VIRULENCE FACTORS OF ENTEROVIRULENT *ESCHERICHIA COLI* (EVEC).

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Samples of raw beef (n=101) and raw milk (n=101) were tested using multiplex polymerase chain reaction assay to determine the incidence of the virulence factor genes of the four main types of EVEC viz. enteropathogenic, enterotoxigenic, enteroinvasive and entero-hemorrhagic/verotoxigenic. The results of the beef survey showed that one sample possessed the 'attaching and effacing' (*eae*A) gene and three possessed the gene for verotoxin 2 (VT2). The milk survey showed that one sample possessed the *eae*A gene and six possessed the VT2 gene.

LONGITUDINAL STUDY OF *E. COLI* O157 ON FOUR DAIRY FARMS IN WISCONSIN. V23/II

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A 14-month longitudinal study was conducted on four dairy farms (C, H, R, and X) in Wisconsin to ascertain the source(s) and dissemination of *E. coli* O157. A cohort group of 15 heifer calves from each farm were sampled from birth to a minimum of 7 months of age (range 7-13 months). The cohort heifers and other randomly selected cattle from farms C and H tested negative. Farm R had two separate outbreaks of *E. coli* O157 lasting 4 months and 1 month in duration while farm X had at least one positive heifer for a 9-month period. *E. coli* O157 was also isolated from other cattle, feed, flies, pigeon, and water associated with the cohort heifers. The number of positive cattle increased following detection of O157 strains in animal drinking water. *E. coli* O157 was found in water at <1 CFU/ml to 50 CFU/ml. Positive heifers shed O157 strains in feces for 1-8 weeks at levels ranging from 200 to 8.7×10^4 per gram. When negative heifers were co-mingled with positive cattle, shedding of *E. coli* O157 occurred in as little as one week post-grouping. These data further highlight contaminated water and grouping as important factors in the dissemination of *E. coli* O157 among dairy cattle.

HIGH OCCURRENCE OF *ESCHERICHIA COLI* SHIGA TOXIN (Stx)GENE SEQUENCES IN HEALTHY CATTLE AT RIO DE JANEIRO, BRAZIL, USING PCR V25/II

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Fecal samples of 121 healthy animals from 10 dairy farms were examined for *stx* gene sequences by PCR. Identity of the amplification products was confirmed by dot blot hybridization with *stx1* and *stx2* probes. Isolation of *Stx*-producing *Escherichia coli* (STEC) from PCR-positive samples were based on colony blotting and hybridization with *stx* probes. For presumptive isolation of 0157:H7 *E.coli* the Tellurite-Cefixime Sorbitol McConkey Agar (TC-SMAC) was used. A high frequency (82%) of *stx* sequences was found. Most samples (69%) harbored both *stx1* and *stx2* sequences, while only *stx1* or *stx2* occurred in 19% and 12% of the samples, respectively. Five *stx1* non-0157 colonies from 2 PCR-positive samples were already isolated and were cytotoxic for Vero cells. The use of TC-SMAC allowed the isolation of 17 0157:H7 colonies from 2 calves at different farms. These isolates were *stx2* probe-positive, but no cytotoxic activity was observed in Vero cells. This is the first report of isolation of 0157:H7 *E.coli* in Brazil. The high frequency of *stx* sequences in bovine feces is probably related to the high occurrence of STEC described in beef products at Rio de Janeiro.

V29/II

DYNABEADS® PLUS 3M PETRIFILM-HEC®: AN IMPROVED SCREENING METHOD FOR DETECTION OF *E. COLI* O157 FROM MINCED BEEF

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Immunomagnetic separation (IMS) technique (Dynabeads®, Dynal, N) resulted in improved sensitivity of detection, when screening meat products for *E. coli* O157. The number of sorbitol non-fermenting microorganisms other than *E. coli* O157 that adhere non-specifically to the magnetic beads hampers the application of this method. We combined IMS with 3M Petrifilm-HEC® (3M Company, MN). After enrichment in „m-EC + n" medium (42°C for 6 h) and IMS followed by subculture to 3M Petrifilm (42°C for 18 h), the Petrifilm® plates were tested for the presence of the O157 antigen using the Petrifilm® HEC direct blot ELISA. Samples giving positive disc blots were then subjected to further analysis. This combination limits the number of false positive results. Detection sensitivity was approx. 20 CFU/g artificially inoculated minced beef. Testing this method on 153 samples of minced beef (incl. 21 naturally contaminated) sensitivity was 100% and specificity 80.3%. The Vitek Immunodiagnostic Assay System (VIDAS E. coli O157; bioMérieux, F) gave 19% sensitivity. and 85.6% specificity, the VIP® (BioControl Systems, WA) 31.3% sensitivity and 86.4% specificity. The combination of IMS with 3M Petrifilm-HEC® is a fast and efficient screening procedure for *E. coli* O157 in minced beef and mitigates some of the problems of IMS.

V31/II

EPIDEMIOLOGICAL AND GENETICAL RELATIONSHIPS BETWEEN SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) ISOLATED FROM SINGLE POPULATIONS OF CATTLE AND SHEEP

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Two separate populations of cattle (N=19) and sheep (N=25) were investigated for Shiga-Toxin (Stx) producing *E. coli* (STEC) over a 6-months period. STEC were isolated from 63.2% of cattle and 88.0% of sheep. STEC from cattle and sheep were heterogeneous for their serotypes and ETs. Some types types of STEC were predominating in cattle (O116:H21, ET14) and others in sheep (O125, ET4; O128:H2, ET11 and O146:H21, ET14). All other STEC types occurred only sporadically. In contrast to their diversity, STEC from distinct animal populations were very similar for their stx-genotypes. Genetic rearrangements in closely related STEC O146:H21 strains could be associated with altered chromosomal locations of stx_1 and stx_2 genes. Our results are indicating that stx-encoding bacteriophages might be the origin of the genetic heterogeneity in STEC from animals.

EVALUATION OF VIDAS ™ *E.COLI* O157 FOR DETECTING OF *ESCHERICHIA COLI* O157 IN NATURALLY CONTAMINATED FOOD SAMPLES

V40/II

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An automated enzyme linked fluorescent immunoassay (ELFA) VIDAS™ *E. coli* O157 was compared with immunomagnetic separation followed by culture to cefixime tellurite Mac Conkey agar (CTSMAC) for detecting *E. coli* O157 in more than 300 naturally contaminated food samples including raw milk cheeses, poultry, raw sausages and ground beef retail samples. Confirmation of the positive samples by ELFA was performed by automated immunoconcentration system VIDAS ICE which allows selective capture and release of target organisms.

THE OCCURRENCE OF VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* O157 ON DAIRY HERDS IN THE NETHERLANDS

V61/II

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To determine the occurrence of O157 VTEC on dairy herds in the Netherlands, 10 farms were visited from September through December 1996. The farms (randomly selected) were located in the northern and eastern part of the country, where dairy herds are most concentrated. Fecal samples were collected from all cows, heifers, yearlings, weaned calves, and preweaned calves present. Following enrichment in modified *E. coli* broth with novobiocin (mEC+n) and immunomagnetic separation of *E. coli* O157, the samples were inoculated onto sorbitol MacConkey agar supplemented with cefixime and tellurite (CT-SMAC). The proportion of animals infected varied from 0-22.4%. Seven farms were identified as O157 VTEC-positive. O157 VTEC were recovered from 34 (7.4%) of 458 cows, from 3 (1.4%) of 218 heifers, from 5 (2.7%) of 186 yearlings, from 29 (14.7%) of 198 weaned calves, and from 4 (4.7%) of 85 preweaned calves. These results confirm that healthy cattle are a reservoir of O157 VTEC, and that the infection rate is highest among weaned calves. Currently, follow-up visits are being performed.

V62/II

VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* O157 IN FECES OF DUTCH VEAL CALVES AND ADULT CATTLE

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Both in 1995 and 1996, feces of dutch veal calves and adult cattle were examined for the presence of O157 VTEC. The samples were collected at the main slaughterhouses of the Netherlands (located at different places in the country), weekly from July until November. Following selective enrichment in modified *E. coli* broth containing novobiocin (mEC+n), the feces were plated onto sorbitol MacConkey agar (SMAC) supplemented with cefixime and tellurite (CT-SMAC) both directly and after incorporation of an immunomagnetic separtion (IMS) step of *E. coli* O157. In 1995, O157 VTEC were isolated from one (0.5%) of 183 fecal samples of veal calves and from 30 (11.1%) of 270 fecal samples of adult cattle. In 1996, O157 VTEC were isolated from one (0.1%) of 214 veal calves and from 27 (10.0%) of 270 adult cattle. The IMS method proved to be far the most sensitive method. Among the 59 isolates, twelve different phage types were identified, types 8 (18.6%), 14 (15.3%) and 31 (11.9%) being the most common. A number (16.9%) of strains could not be phage typed. Dutch cattle appeared to be an important reservoir of human pathogenic O157 VTEC.

V63/II

REDUCTION OF *ESCHERICHIA COLI* O157:H7 IN DAIRY CATTLE BY SELECTED PROBIOTIC BACTERIA

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Fifteen cannulated calves were studied to determine the efficiency of selected probiotic bacteria on reducing/eliminating carriage of *E. coli* O157:H7 (O157). Of 9 control calves administered O157 only, O157 was detected intermittently in rumen samples of all animals throughout 3 weeks postinoculation, and was shed at various levels in feces continuously throughout the experiment (mean 28 days). O157 was isolated from the rumen and colon of 8 of 9 and 9 of 9 calves, respectively, at the termination of the study. Six calves received orally probiotic bacteria (10^{10} CFU) followed 2 days later with O157 (10^{10} CFU). O157 was detected in the rumen for only 9 days postinoculation in two animals, for 16 days in one animal, for 17 days in two animals and for 29 days in one animal. O157 was detected in feces for only 11 days postinoculation in one animal, for 15 days in one animal, for 17 days in one animal, for 18 days in one animal, for 19 days in one animal, and 29 days in one animal. Results indicate that selected probiotic bacteria can reduce the carriage of O157 by cattle when administered before exposure to O157.

INTERACTION BETWEEN *ESCHERICHIA COLI* O157:H7 AND FOOD SPOILAGE BACTERIA

V65/II

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A novel selective plating procedure was used to enumerate *Escherichia coli* O157:H7 from raw minced beef samples inoculated with a three strain mixture of this pathogen and stored in both aerobic and vacuum packed environments. Comparison of the resultant growth characteristics of this pathogen with those observed in control samples, comprising minced beef previously sterilized by irradiation, demonstrated that in vacuum packed samples stored at 15°C, the natural meat microflora inhibited the growth O157:H7. Incubation of this pathogen in raw meat environments was shown to enhance its ability to grow in acidified laboratory media.

VIRULENCE COMPARISON OF *ESCHERICHIA COLI* O157:H7 STRAINS FROM BOVINE AND HUMAN ORIGIN

V66/II

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The virulence of ten O157:H7 strains from healthy cattle and ten strains from multiperson disease outbreaks were compared. All strains were DNA probe-positive for *slt-I* and *slt-II*, and *eae*, and all were positive in the Vero cell cytotoxicity assay. Five 1-day-old gnotobiotic pigs were inoculated per os with each bacterial strain, and observed for clinical signs of diarrhea, anorexia, depression, and signs of central nervous system disease for 8 days or until they became debilitated, then euthanatized and subjected to necropsy and histologic examination. The clinical and pathological results show significant virulence differences in outcomes between bovine-origin and human-origin strains. The results of this study suggest that the gnotobiotic piglet model is a predictor of virulence of O157:H7 *E. coli* strains for human beings, and possibly a predictor of strains which pose the greatest risk for causing HUS. The 20 strains used were also evaluated for SLT production with a quantitative verotoxin assay. These toxin production results were compared with the results of the gnotobiotic virulence ranking to assess in vitro and in vivo virulence similarities.

V71/II CELL DENSITY DEPENDENT ACID SENSITIVITY IN *ESCHERICHIA COLI* O157:H7

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Bacterial ability to survive in highly acidic gastric environment plays a crucial role in food and waterborne diseases. *Escherichia coli* O157:H7, the causative agent of hemolytic uremic syndrome and hemorrhagic colitis, is recognized as a major foodborne pathogen. Importance of this pathogen in public health has been underscored by several recent outbreaks including one in Japan involving about 8000 people. Strains of *E.coli* O157:H7 have been shown to be very resistant to low pH (pH 2-3). The high level of acid resistance of these organisms have been implicated in their ability to survive in acidic foods (apple juice, mayonnaise etc.) and their low infective dose (10-100 CFU). The high level of acid resistance of stationary phase cultures of *E.coli* O157:H7 was found to be dependent on the cell density. At high cell density, the culture was 3-4 log more sensitive than the same culture diluted 100-1000 fold. This cell density dependent acid sensitivity (CDDAS) was mediated by a readily diffusible low molecular weight substance produced in stationary phase. The active substance increased acid sensitivity of several gram-negative and gram-positive bacteria raising the possibility that this substance might be useful in controlling acid tolerance of foodborne pathogens. Although mutation in the *rpoS* gene made cells more sensitive to acidic pH, it completely eliminated the CDDAS.

V72/II DIETARY INFLUENCES ON THE SHEDDING OF *Escherichia coli* O157:H7 BY RUMINANTS.

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Pre-harvest dietary management may play a role in reducing the incidence of *E. coli* O157:H7-positive ruminants. We investigated the effect of: i) two diets with opposite nutritional qualities (grass-hay, G, and corn/pelleted alfalfa, C), ii) an abrupt diet change and iii) fasting on the shedding of *E. coli* O157:H7. Feces were cultured both by a non- and a selective- enrichment protocol, from sheep experimentally inoculated with *E. coli* O157:H7. Sheep fed G shed the bacterium almost twice as long and in higher numbers than sheep fed C. Increased shedding was observed when the diet was abruptly changed from C to G while the opposite dietary change (G to C) decreased shedding of *E. coli* O157:H7. In this study, a 24 hr fast did not influence *E. coli* O157:H7 shedding. Similar effects of diet on the shedding of *E. coli* O157:H7 by cattle will also be presented.

RAPID AND SPECIFIC FLUOROGENIC PCR-BASED SYSTEM FOR THE DETECTION OF SHIGA-LIKE TOXINS I AND II PRODUCING *E. COLI*

V73/II

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Escherichia coli strains which produce Shiga-like toxins I and II have been responsible for major food-borne outbreaks. A rapid and specific fluorogenic PCR-based detection system has been developed for Shiga-like toxin producing *E. coli* (SLTEC) to avoid tedious and lengthy post-PCR detection and enable initial template quantification. This novel PCR assay exploits the endogenous 5' nuclease activity of AmpliTaq™ DNA polymerase which cleaves the fluorogenic probe that hybridizes to a target site between the PCR primers during amplification. This cleavage results in an increase in fluorescence which can be measured quantitatively and scored test samples for the presence of SLT genes. More than 100 SLTEC and 50 non-pathogenic *E. coli* or common enteric bacteria have been tested using our SLT-I and II assay systems and the results were highly correlated with those obtained using independent methods such as Vero cell cytotoxicity or immunological tests. These systems are robust and powerful providing a fast and specific mechanism for the detection of SLTEC in food and environmental samples.

A TWO YEAR STUDY OF *ESCHERICHIA COLI* O157 IN CATTLE, PIGS, SHEEP, POULTRY AND RETAIL MEAT PRODUCTS

V100/II

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Faecal samples from 4800 cattle, 1000 pigs, 1000 sheep and 1000 chickens were collected over a one year period and examined for *E.coli* O157 by immunomagnetic separation and culture onto cefixime tellurite sorbitol MacConkey agar. Strains were characterised by phage type, plasmid profile, toxin genotype, *eaeA* gene and H antigen. Strains of *E.coli* O157 (almost 100 subtypes) were isolated from 15.7% of cattle with a monthly prevalence which varied from 6 to 30%. VT^+ *E.coli* O157 was isolated from 2.2% of sheep but not from either pigs or chickens. In the second year of the study 2062 samples of raw retail processed meats have so far been examined as above. Despite the prevalence in cattle being much higher than in sheep, *E.coli* O157 was isolated from 5.9% of lamb products and 1.5% of beef products, with the highest prevalence (7.5%) being in lambburgers. The study is ongoing and work is in progress to try to explain this higher prevalence in lamb products.

V129/II PREVALENCE OF *ESCHERICHIA COLI* O157 IN MEAT IN DENMARK

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In a nationwide survey 2112 retail samples of minced beef (1584) and pork (528) were investigated for *Escherichia coli* O157. The samples were analyzed by the use of enrichment, immunomagnetic separation (1608 samples) and sorbitol MacConkey agar containing cefixime and tellurite. Seven *E. coli* O157 strains were recovered from seven samples (0,3%). Four strains, two from beef (H7 and H-) and two from pork (H7 and H-) were shown to produce verotoxin (VT) by a vero cell assay. PCR analysis revealed that one strain was VT1 and four strains were VT2 positive. The VT positive strains possessed the *eae* gene and a 60 MD plasmid. The VT negative O157 strains originated from pork (2) and beef (1) (H- and Hro). The VT positive pork samples were demonstrated to contain a bovine serum protein by ELISA. In this survey it is established that *E. coli* O157: H7/H- can be isolated from minced meat in Denmark and that other minced meat types than beef may play a role as source of infection due to possible cross contamination at the retail level. The VT positive *E. coli* O157 exhibited the expected virulence markers whereas the VT negative did not.

V154/II EFFECTS OF THE RUMEN MICROENVIRONMENT ON THE GROWTH AND FECAL SHEDDING OF *E. COLI* O157:H7

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The effects of fasting on the rumen microenvironment and on the ruminal proliferation and fecal shedding of *E. coli* O157:H7 were studied. Nine calves were fitted with rumen cannulas and inoculated with 10^{10} CFU of *E. coli* O157:H7. Ruminal volatile fatty acids (acetic, propionic, and butyric) concentration and pH, along with *E. coli* O157:H7 populations in the rumen and feces, were monitored before, during, and after two 48-hour fasts. In all calves, fasting decreased the ruminal concentration of volatile fatty acids (VFAs) and increased ruminal pH. While daily ruminal and fecal *E. coli* O157:H7 numbers did not directly correlate with daily ruminal VFAs or pH, fasted calves had lower average ruminal VFAs and larger populations of *E. coli* O157:H7 shed in the feces than did nonfasted control calves. These results suggest that changes in the enviroment of the gastrointestinal tract induced by fasting, such as decreases in ruminal VFA concentrations, may increase fecal shedding of *E.coli* O157:H7.

ESTIMATED ANNUAL COSTS OF *ESCHERICHIA COLI* 0157:H7 DISEASE IN THE UNITED STATES

V155/II

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This study updates previously estimated costs of *E. coli* O157:H7 disease for the estimated 10,000-20,000 annual cases and 200-500 associated deaths in the United States. The cost-of-illness method was used and included the estimation of both medical costs and costs of lost productivity. Estimated annual U.S. costs of *E. coli* O157:H7 disease total $381-$913 million (in 1995 dollars). Assuming 80% of these cases are foodborne, foodborne costs total $304-$730 million each year. These foodborne cost estimates represent the maximum benefits that could be achieved by reducing *E. coli* O157:H7 in the U.S. food supply. Cost estimates such are these were used to calculate the benefits of USDA's Hazard Analysis Critical Control Point (HACCP) system to improve the safety of the U.S. meat and poultry.

CHARACTERIZATION OF VEROTOXIGENIC *ESCHERICHIA COLI* FROM RAW AND READY-TO-EAT MEAT PRODUCTS

V171/II

AJ Yee[1]*, A Martin[1], M Rozwadowski[1], S Read[2], K Rahn[2], R Johnson[2], P Johnson[1], D Alves[1], E Todd[2], K Sandu[3] and CL Gyles[3].[1] Ontario Ministry of Agriculture, Food and Rural Affairs, [2] Health Canada, [3] University of Guelph, Ontario, Canada.

Strains of verotoxin-producing *Escherichia coli* (VTEC) isolated from 1,070 raw and ready-to-eat meat products were tested for potential virulence attributes including toxin type, EHEC-hemolysin (EHEC hly), *eae*A and *bfp* genes, a 60 MDa plasmid, and the EAF plasmid. Strains were tested by PCR for VT1, VT2, *eae*A, *bfp*, EAF, and EHEC hly sequences, and plasmid profiles screened for the 60MDa plasmid. Isolates were also tested for EHEC hemolysin activity on washed sheep blood agar. Of the 25 isolates none were O157:H7, although 11 belonged to serotypes associated with human disease, including O5:NM, O22:H8, O91:H14, O91:H21, O91:NM, O103:H2, and O153:H25. One O103:H2 isolate contained the *eae*A gene, none possessed the EAF plasmid or *bfp*, and 12 contained a 60 MDa plasmid. Typical enterohemolysin activity was detected in 11 isolates which were also positive by PCR for EHEC hly sequences. EHEC hly activity was the most consistent virulence marker in serotypes associated with human disease.

V182/II

THE SURVIVAL OF *ESCHERICHIA COLI* 0157 IN MODEL ECOSYSTEMS AND ON SURFACES

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The aim of this work is to investigate how well verocytotoxin-producing *Esch. coli* 0157 survived in soil, water, cattle faeces and on surfaces simulating those used in food manufacturing. The studies were performed using laboratory-based model systems and survival of *Esch. coli* 0157 determined by viable counts on various selective and differential growth media. It was demonstrated that VTEC could survive for more than 150 days in soil cores, and 90 days in cattle faeces. However, survival was much reduced in cattle slurry (20 days) and river water (15 days). When VTEC were deposited onto stainless steel surfaces they remained viable for more than 40 days. Survival was enhanced at temperatures below 20°C. These studies show that VTEC are able to survive for extended periods in soil, water and on surfaces simulating those used in food processing and production. Considering the infective dose for VTEC infection is extremely low their ability to survive adverse conditions has important implications in the spread of this disease.

V186/II

COMPARISON OF METHODS FOR DETECTION OF *ESCHERICHIA COLI* O157 IN RAW MEAT.

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Three culture methods and two immunoassays were compared in a collaborative study involving 10 laboratories. Freeze dried cultures were distributed to the laboratories which sampled and spiked the meat with 50 cfu/25g, 2 cfu/25g and 0 cfu/25g. The same enrichment culture (ECn) was used for all methods. Six hours of incubation were used for direct plating on sorbitol MacConkey agar with cefixime and tellurite (CT-SMAC), immunomagnetic separation (IMS) and plating on CT-SMAC and IMS followed by 3M Petrifilm HEC-testkit^{R}. Incubation in 18-22 hours preceded the TECRA O157 visual immunoassay and the EHEC-TEK kit from Organon Teknika. No statistical difference were observed between the methods at any spiking level. At 50 cfu/25g 90-95% of the samples were detected with any of the methods. At 2 cfu/25g IMS-CT-SMAC showed the lowest detection rate (46%) while most of the samples (60%) were positive by the IMS-Petrifilm. Part of the explanation of the low positive rate at the low spiking level could be absence of *E. coli* O157 in the spiking volume.

DEVELOPMENT AND EVALUATION OF A 24HR METHOD (*E. coli* SELeCT™) FOR THE DETECTION, ISOLATION AND QUANTIFICATION OF *ESCHERICHIA COLI* O157:H7 IN RAW GROUND MEAT

V192/II

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Filtration technology has been applied to develop a rapid diagnostic method (*E. coli* SELeCT™) for *E. coli* O157:H7 that can be used to detect, isolate and quantify less than one cfu/g without prior enrichment. This procedure involves a brief centrifugation step, filtration and resuscitation before presumptive *E. coli* O157:H7 cfu are observable on Rainbow O157 Agar (BIOLOG, Inc). Confirmation is accomplished with a colony lift immunoassay (CLI) using an affinity purified antibody specific for *E. coli* O157:H7 (Bac Trace™, KPL). The mean sensitivity and specificity levels calculated in several experiments approximated 100%. Comparison studies showed the SELeCT™ system to be consistently more accurate and precise than the Most Probable Number (MPN) method for quantifying *E. coli* O157:H7.

FECAL SHEDDING OF *ESCHERICHIA COLI* O157:H7 DURING THE BEEF CATTLE PRODUCTION CYCLE

V199/II

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Fecal shedding of *Escherichia coli* O157:H7 was studied in a closed beef cattle herd in Alberta. There was a significant increase in the fecal isolation rate (FIR) of the organism from cows after parturition and calves after weaning. The highest *E. coli* O157:H7 FIR occurred during the summer among yearling steers fed silage/grain. The FIR for steers changed to an alfalfa hay diet for 3 weeks (0/17) was significantly lower than the FIR for steers that stayed on silage/grain (9/18).

V203/II VIRULENCE FACTORS OF DIFFERENT *E.COLI* SEROTYPES ORIGINATING FROM FOOD OF ANIMAL ORIGIN

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Raw foods of animal origin were examined for the presence of *E.coli* 0157 and other verocytoxin producing (VTEC) strains in the Federal Republic of Germany. Using immunomagnetic separation (IMS) 0157-strains were isolated from raw milk, pork and lamb. The Polymerase Chain Reaction (PCR) showed that none of the three 0157.H$^-$ and two 0157-H16 isolates harboured virulence factors. Further VTEC strains were detected with an enzyme immuno assay (EIA) as screening method, especially on lamb carcases. With the PCR *E.coli* strains with different VT patterns and the *E.coli* attaching and effacing gene (eae) could be detected. Different serotypes (e.g. 010.H4, 070.H11, 0103.H31, 0119.H$^-$, 0156.H$^-$) with full virulence patterns were found, which to the author's knowledge have not been isolated in connection with hemorrhagic colitis (HC) or the hemolytic uremic syndrome (HUS). The question whether further virulence factors play a role in EHEC infection must be adressed.

V204/II DETECTION OF *ESCHERICHIA COLI* O157:H7 IN MINCED MEAT BY IMMUNOMAGNETIC SEPARATION (IMS) IN A MODEL EXPERIMENT

Susanne Heckötter*, Christiane Schuy and Michael Bülte

Reisolation rates of one *E. coli* O157:H7 strain, artificially inoculated into minced beef samples, were established with and without using Dynabeads anti *E. coli* O157 for immunomagnetic separation (IMS) under different methodical conditions. Independent of the level of contamination, the IMS was superior to the control procedure; good results could be found even after a 6 hour incubation. The best results were obtained by enriching in parallel using 2 different enrichment media for 6 and 24 hours followed by IMS and subcultivation on two selective media. The suitability for use on frozen samples was evaluated. The results indicate that the IMS is a sensitive, convenient and rapid method for the isolation of *E. coli* O157 in foods. This method can be recommended as a german standard (DIN [Deutsches Institut für Normung] -Norm) method.

VERYOCYTOTOXIN EXPRESSION IN FOOD

V205/II

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Protein fusions have played a central role in molecular genetic studies of protein expression and export from bacterial cells. Conventionally this approach involves the fusion of a selectable "reporter" gene which possesses an easily assayable activity, to the promotor region of the gene under study. As a simple and sensitive means of monitoring the expression of verocytotoxin the transposon TnphoA was used to create a fusion between the A subunit of the cloned slt-I operon and the gene for bacterial alkaline phosphatase. This fusion was introduced into Escherichia coli CC118. This study describes the data from experiments in which the slt::TnphoA gene fusion was used to ascertain which cultural/environmental parameters influenced the expression of VT1 and VT2 in tap water, milk and raw meat. The expression of these toxins by E.coli 0157:H7 under similar cultural conditions was ascertained by high performance liquid chromatography. In this study it has been observed that VT1 expression increases markedly when the above bacterial cultures are grown in tap water i.e. under conditions of nutrient stress. No such response was evident in milk.

SHIGA-LIKE TOXIN-PRODUCING *ESCHERICHIA COLI* IN FOODS

V210/II

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Foods are the most frequently implicated source of *Escherichia coli* O157:H7 and other Shiga-like toxin-producing *E. coli* (STEC) infections in outbreaks and sporadic cases. Although raw ground beef and other red meats are considered the highest risk foods, processed meats, milk, salad dressings, apple cider, vegetables, poultry meats and water may also be vehicles of STEC. Food contamination occurs principally through contact with manure of animal reservoirs, with manure-contaminated water or with other contaminated foods or equipment. Domestic and wild ruminants are the most frequent carriers of STEC. The more virulent STEC, such as *E. coli* O157:H7 are less prevalent in animals and foods than other STEC. However, some outbreak strains of STEC have properties such as increased acid resistance which suggest they are better able to survive in the food chain than other STEC and may have greater virulence for humans. Such properties may reflect in part a rapid adaptive response in these organisms associated with the high mutator phenotypes identified recently in pathogenic strains of *E. coli* O157:H7 and Salmonella.

V214/II

ISOLATION OF VEROTOXIN-PRODUCING *ESCHERICHIA COLI* O157 : H7 FROM BEEF AND PORK IN CHANGCHUN, CHINA

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A tolal of 70 samples of retail beef and pork from Changchun,China was assayed for the presence of *Escherichia coli* serogroup O157 : H7 by a multiple procedure. The procedure involves several steps, including selective enrichment, sorbitol-MacConkey agar (SMAC) culture, biochemical profile, slide and tube agglutination test and polymerase chain reaction (PCR). *Escherichia coli* O157 : H7 was isolated from 2 (5%) of 40 beef and 1 (3.3%) of 30 pork samples.

V215/II

RAPID AND SPECIFIC DETECTION OF VEROTOCIN-PRODUCING *ESCHERICHIA COLI* BY THE POLYMERASE CHAIN REACTION

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Two sets of synthetic oligonucleotide primers derived from sequences of the VT1 and VT2 genes were used in a polymerase chain reaction (PCR) amplification procedure to detect these genes in some enteric pathogens. A total of 4 verotoxin-producing *Escherichia coli* strains and 41 other recognized pathogens were studied. PCR amplification products identifying the VT1 and VT2 gene sequences were observed only in DNA extracted from strains found to be VT positive in traditional tissue culture assays.the primers were clearly able to distinguish VT1, VT2 and VT1-and VT2-producing strains of *Escherichia coli*.Template DNA extracted from other enteric pathogens was found to be negative with the excepion of 1 strain of *Shigella dysenteriae* type 1 in which good amplification with the VT1 primer was observed.The sensitivity of the PCR procedure for detection of both VT1 and VT2 genes was determined to be 320pg of total-cell DNA. Furthermore, the VT1 gene was easily detected when only 32pg of DNA was used as the template in the PCR procedure.

PREVALENCE OF STEC IN ANIMAL FAECES AT SLAUGHTER V227/II

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Ruminants are a major reservoir of STEC and their meat a vehicle entry of STEC into the human foodchain. We determined the incidence of STEC shedding by animals pre-slaughter and their virulence characteristics. Faecal enrichments were screened using an *stx* PCR, followed by isolation and testing isolates for *stx*, *eae* and EHEC plasmid. Faeces from 576 animals were tested of which 30% were *stx* PCR+, STEC were isolated from 31% PCR+ samples, the overall isolation rate being 9%. The highest rate was from lambs and calves, followed by grass-fed and grain-fed cattle, respectively. The additional virulence factors were detected least often in lambs and most often in calves. O157 was isolated from 1.7% of animals and was not evenly distributed. These results suggest age and diet affect shedding of STEC by animals pre-slaughter.

DYNAMICS OF STEC and *E. coli* IN FEEDLOT CATTLE V228/II

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CSIRO Division of Food Science & Technology and The University of Queensland, Brisbane, Australia

Understanding the factors eg. diet and stress, influencing STEC shedding in cattle and how they can be managed will contribute to reducing the incidence of STEC on beef. The shedding of *E. coli* and STEC was followed in a mob of feedlot cattle, from induction to the final ration pre-slaughter. The shedding of STEC was found to be higher at induction than at slaughter and shedding of total *E. coli* and STEC fluctuated during the initial feed changes before stabilising when the animals were on their finishing ration. STEC isolates were compared using PFGE. At induction, multiple, distinct PFGE types were isolated; however, as residence time in the feedlot progressed, two similar types dominated. These findings have implications for the management of cattle prior to slaughter.

V233/II **INOCULATION OF WHITE-TAILED DEER WITH *E. COLI* 0157:H7**

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Experimental *E. coli* O157:H7 infection was studied in white-tailed deer. Six 3-month-old deer were orally inoculated with 10^8 *E. coli* O157:H7, 2 received non-toxigenic *E. coli*, and 2 received no inoculum. Inoculated deer were shedding 10^3 to 10^5 CFU per gram of feces by 1 day post inoculation (PI), but remained clinically normal during the trial. Fecal shedding of *E. coli* O157:H7 decreased dramatically by 2 weeks PI but remained intermittently detectable throughout the 4-week trial. To assess contact transmission, an uninoculated deer was placed with an inoculated deer at 12 days PI. Fecal shedding by the introduced deer was detected within 2 days. *E. coli* O157:H7 was recovered from the gastrointestinal tracts of all inoculated deer necropsied at intervals throughout the trial. Significant lesions were not apparent in any deer. The results of this study are similar to those of experimental studies in bovine calves.

V236/II A SURVEY OF RAW BEEF AND RAW MILK FROM NORTHERN IRELAND FOR THE VIRULENCE FACTORS OF ENTEROVIRULENT *ESCHERICHIA COLI* (EVEC).

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Samples of raw beef (n=101) and raw milk (n=101) were tested using multiplex polymerase chain reaction assays to determine the incidence of the virulence factor genes of the four main types of EVEC; enteropathogenic, enterotoxigenic, entero-invasive and enterohemorrhagic/verotoxigenic. The results of the beef survey showed that one sample contained an *E. coli* isolate possessing the 'attaching and effacing' (*eaeA*) gene and three samples contained *E. coli* isolates possessing the verotoxin 2 (VT2) gene. The milk survey results showed that one sample contained an *E. coli* isolates possessing the *eaeA* gene, eight samples contained *E. coli* isolates possessing the VT2 gene and one sample contained two *E. coli* isolates, one possessing the *eaeA* gene and one possessing VT2 gene.

BINDING OF VEROTOTOXIN TO PORCINE GLOMERULI AND TO PROTEIN RECEPTORS ON THE SURFACE OF VERO CELLS

V12/III

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Frozen kidney sections of several animal species were examined to assess which species may be useful as a model for verotoxin (VT) mediated HUS. The pig was the only species in which VT bound to the glomeruli and binding was blocked by treatment with formaldehyde. A blocking effect was also shown for VT binding to Vero cells. These findings led us to the hypothesis that VT binds to a cell surface protein. When Vero or VRP (Gb_3 deficient Vero) cells were incubated with ^{125}I-labelled VT2 and DSS X-linker, SDS-PAGE of cell lysates showed labelled bands at 44, 50, 60, 86, 102 and 138 kDa. When ^{125}I-labelled VT1 was cross-linked, radioactive bands occurred at 51, 67, 101, 160, 188, and 232 kDa but when ^{125}I-labelled VT1 B subunit was used, only a single band at 50 kDa was observed. CHO cells did not bind labelled VT. Binding isotherms revealed that VT1 and VT1 B subunit showed positive cooperativity between at least two binding sites but VT2 binding fit a single class of binding sites. These results indicate that VT binds to protein(s) on the surface of susceptible cells and this binding is different between VT1 and VT2. These studies also suggest that a second receptor, other than Gb3, may be important in the biological activity of VT.

VIRULENCE OF SHIGA TOXIN-PRODUCING *E. COLI* (STEC) IN A MOUSE MODEL CORRELATES WITH TOXIN ACTIVATION

V15/III

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The oral LD_{50} of STEC strain B2F1, which produces two Stx2 variant toxins, is less than 10 organisms in streptomycin(str)-treated mice. In contrast, the oral LD_{50} of the Stx2c-producing O157 strain E32511/HSC is 10^{10} bacteria. Additionally, the Stxs produced by B2F1 are activated 10-1000-fold for Vero cell toxicity by preincubation with mouse or human intestinal mucus, whereas Stx2c of E32511/HSC is not activatable. In this study, we fed three additional STEC that produce Stx2-type toxins to str-treated mice and found that only the two strains with activatable toxins were mouse virulent. We are currently testing the hypothesis that the mouse virulence of an STEC strain correlates directly with the capacity of its Stx2 variant toxin to be activated. We are creating, through allelic exchange, an E32511/HSC strain that encodes an activatable toxin gene in place of *stx*2c. We will assess this modified E32511/HSC strain for mouse virulence. Additionally, since the Stxs produced by B2F1 and E32511/HSC differ by only two amino acids in the A subunit, we are altering the two amino acids in the activatable Stx2 variant to the corresponding amino acids in Stx2c so as to determine which one(s) of these amino acids are required for activation.

V16/III

STRUCTURE-FUNCTION ANALYSIS, PURIFICATION, AND IMMUNOREACTIVITY OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* INTIMIN

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The intimin protein (EaeA), encoded by *eaeA,* is required by Enterohemorrhagic *Escherichia coli* (EHEC) to form attaching and effacing lesions. The aims of this work are to define the minimum region of intimin required for HEp-2 cell binding and to generate intimin-specific reagents. Evidence from our laboratory and others suggests that amino acids critical for intimin-mediated adherence are contained within the C-terminal portion of the protein. We constructed various His-tagged intimin derivatives which will be tested for complementation of an *eaeA* deletion mutant in adherence assays. Anti-intimin monoclonal (mAbs) and polyclonal antibodies are also being generated. We will assess the capacity of these antibodies to block EHEC adherence. Purification of several His-tagged intimin derivatives yielded mg quantities of protein. The derivatives that have been tested were acid stable. Purified intimin fragments, mAbs, and monospecific Abs will be used to develop intimin-based vaccines and detection kits.

V17/III

INCREASED LEVELS OF INTRACELLULAR CALCIUM ARE NOT REQUIRED FOR THE FORMATION OF ATTACHING AND EFFACING LESIONS BY ENTEROPATHOGENIC (EPEC) AND ENTEROHAEMORRHAGIC ESCHERICHIA COLI (EHEC)

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The aim of this study was to characterise the precise temporal and spatial nature of the previously reported rise in intracellular calcium concentration $[Ca^{2+}]_i$ seen in EPEC and EHEC-infected cells. $[Ca^{2+}]_i$ measurements were made in EPEC and EHEC infected HEp-2 cells loaded with the fluorescent ratiometric dye fura-2 using digital fluorescence microscopy and dedicated calcium imaging software. Using both rapid and gradual infection procedures during which extensive attaching & effacing (A/E) lesion formation occurred, we were unable to detect any significant increases in $[Ca^{2+}]_i$ in cells infected with 4 classical EPEC and 2 EHEC strains; large (up to 10-fold) increases in $[Ca^{2+}]_i$ were detected at the end of each experiment however, when infected cells were exposed to either histamine or to the calcium ionophore, ionomycin. Furthermore, chelation of $[Ca^{2+}]_i$ with BAPTA prior to cell infection did not affect the ability of bacteria to form A/E lesions. These results contradict earlier studies and indicate that $[Ca^{2+}]_i$ in HEp-2 cells are not affected by infection with either EPEC or EHEC strains and that rises in $[Ca^{2+}]_i$ are not required for A/E lesion formation.

DOWN REGULATION OF INTIMIN EXPRESSION DURING ATTACHING & EFFACING ADHESION

V18/III

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Enteropathogenic (EPEC) and enterohaemorrhagic *E. coli* (EHEC) produce attaching & effacing (A/E) lesions in the intestinal mucosa. Intimate bacterial adhesion associated with A/E lesion formation is promoted by intimin, a 94 kDa surface protein. Anti-intimin α and anti-intimin β antisera were employed in immunolabelling studies to investigate the expression of intimin α by EPEC strain E2348/69(O127:H6) and intimin β by EHEC strain 3605-73(O26:H11). Following a 3 h incubation of HEp-2 cells with EPEC and EHEC, double immunofluorescence labelling of intimin and cellular actin revealed strong intimin expression by A/E bacteria but by 6 h incubation intimin expression by most bacteria was reduced or not detected. This down-regulation of intimin expression following A/E lesion formation was not observed with strain JPN15, a virulence plasmid-cured derivative of E2348/69, but was partially restored when a plasmid harbouring the virulence plasmid-encoded regulatory *(per)* genes was introduced. These results indicate that surface expression of intimin is regulated following A/E lesion formation and that, in the case of EPEC, virulence plasmid-encoded genes participate in this process.

THE NEW VEROTOXIN ENCODING BY PHAGE FROM *ESCHERICHIA COLI* ASSOCIATED WITH SWOLLEN-HEAD SYNDROME IN CHICKENS

V21/III

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A total of 72% of *Escherichia coli* strains isolated from chickens with Swollen-Head Syndrome (SHS), produced a cytotoxin related to Shiga-like toxin (SLT), active on Vero and HeLa cells. However, these strains of SHS-*E. coli* were not hybridized by DNA probes for SLT-I and SLT-II. The SHS-cytotoxin was purified and compared some of its properties with SLT-I and SLT-II. This cytotoxin is also associated with a temperate toxin-converting bacteriophage. *E. coli* K12C600 acquired the ability to produce SHS-cytotoxin after lysogenization by phage isolated from strains *E.coli* -SHS.

V24/III

VIRULENCE MARKERS AMONG SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) STRAINS ISOLATED FROM ANIMAL AND HUMAN SOURCES IN BRAZIL

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Thirty one non-0157 STEC strains isolated from beef products (21), cattle (5) and humans (5) were assayed for adherence to HeLa, HEp-2 and Caco-2 cells, production of hemolysins, fluorescent actin-staining (FAS) test and presence of *eae*A, EHEC (CVD419) and α-hemolysin genes. In addition, 10 O157:H7 colonies harboring *stx* 2 genes and isolated from fecal samples of 2 healthy calves were also studied. Difuse or aggregative adherence patterns were found in 14.3% of the strains from beef samples. Localized adherence was observed in most humans (4/5) and in some animal strains (4/7) including those of serotype O157:H7, and some of these strains were also *eae*A and FAS positive. Production of enterohemolysin and hybridization with pCVD419 was found in 75% of the strains studied, while only 2 strains were α-hemolytic. Several strains were able to invade Caco-2 cells in aminoglycoside exclusion assays. Invasion was inhibited by cytochalasin D and intracellular bacteria was visualized by electronmicroscopy. Invasion of enterocytes, a previously unrecognized virulence feature of STEC, may be useful for the persistence of these strains in their hosts.

V26/III

SELF-INHIBITION OF N-GLICOSIDASE ACTIVITY OF SHIGA TOXIN

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The A subunit of shiga and vero toxins contains a site of specific N-glicosidase activity. According to X-ray crystallography, the active site of shiga toxin is blocked by C-terminal end of the A chain. Separation of the interacting sites by means of proteolytic cleavage and chemical modification of the disulfide bond leads to 50-fold increase in shiga toxin activity in a cell-free system. The mechanism of this effect was investigated in full detail by means of site-directed mutagenesis.

IDENTIFICATION OF A COMMONLY OCCURRING *E. COLI* CHROMOSOMAL DNA-SEQUENCE WHICH IS SPECIFICALLY ABSENT IN STEC O157, O145 AND EPEC O55 STRAINS

V32/III

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Among Shiga-Toxin producing *Escherichia coli* (STEC), strains of serogroup O157 are the most virulent for humans. The reason for the virulence differences between different STEC serotypes is not completely understood. We were interested in hitherto unknown factors which differentiate E. coli O157 from other STEC. By cloning chromosomal DNA of a fecal E. coli O6:H- strain negative for all known virulence markers of STEC, we developed a 1.3 kb gene-probe (pEO67) which did not react with STEC of O-groups 157 and O145, nor with *E. coli* O55, the presumptive ancestor of STEC O157. However, the probe reacted with 120 tested other human pathogenic and apathogenic *E. coli* types including STEC of other serotypes and non-toxigenic O157:H43 strains. Transformation of STEC O157 with cloned pEO67 results in a number of phenotypical alterations including expression of the plasmid-encoded EHEC-hemolysin, therefore possibly affecting virulence.

CELLULAR UPTAKE AND PROCESSING OF SHIGA TOXIN

V36/III

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Shiga toxin intoxicates cells by first binding to GB3 at the cell surface, and then the toxin is endocytosed. In sensitive cells the toxin is transported to the trans-Golgi network and retrogradely to the ER from where translocation may occur. To learn more about the intoxication process we have investigated the importance of amino acids surrounding the cleavage site for furin in the A-chain. Clearly, more amino acids than those known to be involved in recognition by furin are important for toxin processing and toxicity. The A-chain of Shiga toxin contains a disulfide bond which has to be reduced to obtain maximal enzymatic activity. We have studied the stability and the effects of a mutated toxin molecule without the disulfide bond, and the data indicate that the disulfide bond is required both for the stabilization of the toxin and for keeping the subunits together after processing. Sorting of the toxin to the Golgi apparatus and the ER seems to be dependent on the lipid composition of the glycosphingolipids, and recent results suggest that also other lipids in the membrane play a role for efficient intoxication.

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ENHANCED ADHERENCE OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* ISOLATES FROM CASES OF HUMAN DISEASE TO INTESTINAL EPITHELIAL (HENLE 407) CELLS

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We have recently described a large food-bourne outbreak of STEC disease caused by contaminated fermented sausage (J. Clin. Microbiol. [1996] 34:1622-1627). Several serotypes of STEC were isolated from the contaminated food source, but of these, only a subset were isolated from patients with diarrhea or HUS. In the present study we characterized these STEC isolates with respect to presence of putative virulence-associated genes, and capacity to adhere to a human intestinal epithelial cell line (Henle 407). The O111:H$^-$ STEC strain isolated from most of the outbreak HUS patients was shown to adhere in a dose-dependent, mannose-resistant fashion. Confocal microscopy revealed a diffuse pattern of adherence for this, as well as several other STEC strains. Interestingly, the adherence of STEC strains from HUS cases was significantly greater than that of STEC strains found in the contaminated food source, but not in any patients. These studies support the hypothesis that an enhanced capacity to adhere to intestinal cells contributes to the human virulence of STEC.

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ENTEROAGGREGATIVE, SHIGA-TOXIN-PRODUCING *ESCHERICHIA COLI* O111:H2

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Shiga-toxin (Stx)-producing *E.coli* (STEC) O111:H2 strains from an outbreak of hemolytic-uremic syndrome showed aggregative adhesion to HEp-2 cells. They were negative for the attaching and effacing (AE) gene (*eaeA*) and the enterohemorrhagic *E.coli* (EHEC) plasmid markers, but positive for enteroaggregative *E.coli* (EAggEC) probe PCVD432 and the EAggEC heat-stable enterotoxin 1 gene. Hybridization analysis showed that the EAggEC gene cluster was located on a large plasmid while the *stx* gene on the chromosome. The *E.coli* O111:H2 strains described here present a novel combination of virulence factors of both EHEC and EAggEC and might be as pathogenic to humans as the classic EHEC strains are. Besides the EHEC plasmid markers and the characters associated with the AE property, STEC from cattle, beef, and other cattle products should also be examined for EaggEC properties before excluding their pathogenicity to humans.

CHARACTERIZATION OF FIMBRIAE PRODUCED BY ATTACHING-EFFACING *ESCHERICHIA COLI* ISOLATED FROM CATTLE

V64/III

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Thirteen Attaching-Effacing *Escherichia coli* (AEEC) strains isolated from cattle with diarrhea were tested *in vitro* for the presence of adhesive factors. All strains exhibited adhesion on HEp-2 and MDBK cells. Scanning electron microscopy revealed the presence of fimbrial structures. Colony blot hybridization with several DNA probes derived from known *E. coli* virulence genes (EAF, BFP, ...) showed that most strains did not possess homologous sequences. Upon fimbrial extraction from VTEC strain 340S89 (O118:H16), a fimbrial subunit with a mass of 16,5 kDa was separated by SDS-polyacrylamide gel electrophoresis. Internal amino acid sequences of the subunit showed some homology with $F7_2$ fimbriae of human uropathogenic *E. coli.* Purified 340S89 fimbriae as well as antiserum elicited to the purified fimbrial antigen, reduced the capacity of the strain 340S89 to infect MDBK cells. We suggest a role of these surface appendages in the interaction of bovine AEEC with eukaryotic cells, as well as in the pathogenesis of intestinal disease caused by bovine AEEC.

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DIVERSITY OF THE EHEC-HEMOLYSIN A GENE

V69/III

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Diversity of the EHEC-hemolysin A gene (*ehxA*) was examined by restriction mapping analysis of PCR products from 55 verotoxigenic *Escherichia coli* isolates of 33 serotypes. Ten subtypes were identified and none of 11 serotypes with multiple isolates could be split into further subtypes. Estimated rates of nucleotide substitutions, based on the restriction data showed that the *ehxA* subtypes fell into two distinct groups; one with only *eaeA*-positive isolates and the other with only *eaeA*-negative isolates. Sequencing of the *ehxA* gene of a representative of the *eaeA*-negative group showed 98% and 97.3% identity at the nucleotide and amino acid levels, respectively, with the published sequences of the *ehxA* gene of the *eaeA*-positive O157:H7 serotype. All the functionally active domains of the EhxA protein were highly conserved and the two groups probably represent evolutionary markers without functional significance. The data suggest a parallel evolution of both genes without frequent horizontal transfer, or a strong functional relationship between the *eaeA* gene and genes located on the EHEC-hemolysin plasmid.

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VIRULENCE MARKERS OF SHIGA TOXIN PRODUCING ESCHERICHIA COLI (STEC) FROM HEALTHY CATTLE

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Two hundred and seventy-two bovine STEC were examined for *eaeA*, EHEC-hemolysin A (*EhxA*), EAF plasmid and *bfp* sequences; and for production of EHEC-hemolysin (Ehx), adherence to HEp-2 cells and bovine colonocytes, and fluorescent actin staining (FAS) in order to determine the association of these properties with serotypes implicated in human disease. All isolates were negative for EAF and *bfp* sequences. Ehx production and presence of *eaeA* were each highly correlated with serotype; other properties varied among strains. Among 93 *eaeA*-positive isolates, 91 were Ehx-positive and 48 were LA/FAS-positive; among 179 *eaeA*-negative isolates, 62 were Ehx-positive and none was LA/FAS-positive. Twenty of the *eaeA*-negative isolates showed diffuse adherence and five showed aggregative adherence to HEp-2 cells. Adherence to bovine colonocytes was observed with 73% of the *eaeA*-positive and 26% of 120 *eaeA*-negative isolates. Production of Ehx was the marker most highly associated with serotypes of STEC implicated in human disease.

V91/III

COMPARISON OF *ENTEROHEMORRHAGIC E. COLI* AND *ENTEROPATHOGENIC E. COLI* BINDING TO GLYCOLIPID/LIPID RECEPTORS

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Binding of *enterohemorrhagic* and *enteropathogenic E. coli* to glycosphingolipids and phospholipids was compared using thin layer chromatography overlay assays and receptor-based immunoassays. Seven clinical EHEC strains of serotype O157 bound well to gangliotetraosylceramide (Gg_4) and phosphatidylethanolamine (PE) and weakly to gangliotriaosylceramide (Gg_3). This binding was distinct from that of EPEC, a commensal *E.coli* strain. Of the six strains of EPEC tested (including E2348), only two bound Gg_4 and three bound weakly to PE. Postive PE binding strains also bound a species within a HEp-2 cell extract which comigrated with commercial PE on TLC. Binding to PE was temperature-dependent and sensitive to the presence of divalent cations. These findings indicate distinct glycolipid binding specificity for *enterohemorrhagic* and *enteropathogenic E.coli* and may reflect differences in bacterial adhesion to host cell surfaces and/or signal transduction events induced by attachment of these *E. coli.*

LOCALIZATION OF THE BINDING SITE FOR A FLUORESCENT ANALOGUE OF GLOBOTRIAOSYL CERAMIDE IN VEROTOXIN 1 USING FLUORESCENCE SPECTROSCOPY

V92/III

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Verotoxins (VTs) from *Escherichia coli* elicit human vascular disease via specific binding to globotriaosyl-ceramide (Gb_3) receptors located on endothelial cell surfaces. Molecular models based on the crystal structure of VT1 were previously used to investigate the structural basis for receptor recognition by VT1 and related verotoxins. In this study, fluorescence spectroscopy using the VT1 B subunit pentamer was used to test model-based predictions of the location of the Gb_3 binding site. Fluorescence resonance energy transfer (FRET) was used to calculated the apparent distance between a coumarin probe replacing the fatty acyl tail of the Gb_3 glycolipid to the single tryptophan residue (Trp34) present within each VT1 B subunit monomer/receptor complex. The acquired data suggest that these two moieties are approximately 13. 3 Å apart which is consistent with proposed models for the binding of Gb_3 within the "cleft regions" of the VT1 B-subunit. When the proximity of Trp34 residues present on adjacent monomers within the same VT1 B pentameric complexes are taken into consideration, the data suggest that the modified Gb_3 analogue used here associates with the proposed site II receptor binding region of VT1 which was originally identified from molecular modeling studies.

A DIVALENT GALABIOSYL ANALOGUE INHIBITS VT1/Gb_3 BINDING IN VITRO AND PROTECTS CELLS AGAINST VT1 AND VT2

V94/III

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A series of water soluble mono-, di-, tri- and tetravalent galabiose analogues have been synthesized and tested for ability to inhibit VT/Gb_3 binding. The divalent analogues were coupled via linkage of the ßgalactose to benzene in either ortho or meta linkage. The mono, tri and tetravalent analogues, together with most of the divalent analogues were found to be ineffective. However, one species, in which the galabiose moieties were dimerized via the meta position of the benzene ring, showed complete inhibition of VT1/Gb_3 binding at a concentration of 1mM. This analogue was effective to protect vero cells against VT1 cytotoxicity. Less protection against VT2, but no protection against VT2c or VT2e was found. These studies indicate that competitive inhibition of receptor binding may provide an effective mechanism to prevent verotoxin-induced pathology. This inhibitor provides for the first time, an opportunity to study the galabiose/verotoxin interaction by physical methods. The differential protection towards the different VT's may result from different receptor binding site usage by the different VTs as we have previously suggested. The efficacy of the meta, as opposed to the ortho-linked analogue, may relate either to the spacing of the galabiose units or to a differential effect on carbohydrate conformation as we have proposed for lipid-bound galabiosyl species.

V95/III ROLE OF VEROTOXIN-1 RECEPTOR IN THE RETROGRADE TRANSPORT AND SIGNAL TRANSDUCTION OF THE VT B SUBUNIT-LIKE DOMAIN CONTAINING B-CELL RESTRICTED ANTIGEN CD19

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Verotoxin-1 and its binding subunit B alone can undergo retrograde transport to the nuclear membrane via the endoplasmic reticulum after receptor mediated endocytosis and subsequently induce apoptosis. The N-terminal extracellular domain of B-cell specific antigen CD19 has high amino acid sequence similarity to the VT-B subunit and possible lateral association with VT1 receptor globotriaosyl ceramide (Gb_3- also called CD77) on the germinal B-cell surface. Ligation of cell surface CD19 also induces B-cell apoptosis. To characterize the possible role of Gb_3 in CD19 signal transduction, intracellular routing and induction of apoptosis after antibody crosslinking of cell surface CD19 were studied by immunofluorescence, post-embedding immuno-electronmicroscopy and nuclear cytochemical staining in Gb_3^{+ve} and Gb_3^{-ve} Daudi lymphoma cells. The nuclear targeting of CD19 and induction of apoptosis were observed in Gb_3^+ cells only , indicating a central role for Gb_3 dependent retrograde transport of CD19 to the nuclear envelope in CD19 signalling.

V96/III NOVEL APPROACH TO THE SYNTHESIS OF SOLUBLE INHIBITORS OF VT-Gb_3 BINDING

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The receptor function of globotriaosyl ceramide, Gb_3 for the verotoxins is well documented. Although binding is specific for the terminal galabiose disaccharide, VT binding to the free sugar is less, by many orders of magnitude, than to Gb_3 or Gb_3 containing plasma membranes. We have attempted to design a synthetic approach to mimic the effect of the lipid moiety and membrane presentation on Gb_3 carbohydrate/VT receptor function and generate monovalent soluble "glycolipid mimics" which show binding affinities of the order of the native receptor. Our particular approach to design inhibitors was to cleave the double bond of the sphingosine of Gb_3 or lyso-Gb_3 and to couple rigid hydrocarbon units via the carboxylic function generated. To accomplish this a new efficient oxidation method based on $KMnO_4$ was first developed. Thus derived soluble 'globotriaosyl ceramidic acid' from natural Gb_3 and the serine oligosaccharide from lyso-Gb_3 were coupled to rigid hydrophobic frames such as the α-adamantal group. At concentrations within the lower μM range, these novel glycoconjugates show 50% inhibition of VT1 binding to membrane Gb_3 .

Gb_3 FATTY ACID ISOFORM-DEPENDENT VT TARGETING OF THE NUCLEAR ENVELOPE DETERMINES VT-CELL SENSITIVITY

V97/III

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VT sensitivity is markedly increased in multidrug resistance variants of ovarian tumour cell lines, despite similar levels of Gb_3 verotoxin receptor. Similarly, astrocytoma cell lines, show a marked variation in VT sensitivity despite equivalent levels of Gb_3. Using FITC labelled VT1 B subunit, we have shown that in these cells of reduced VT sensitivity, the B subunit is internalized by retrograde transport to the Golgi. However, in the more highly VT sensitive cell lines, the toxin is internalized to the endoplasmic reticulum, nuclear envelope and nucleus. Such intracellular trafficking can be induced in the less sensitive cells by culture in the presence of sodium butyrate. ER/nuclear targeting was found to correlate with the synthesis of an increased level of shorter Gb_3 fatty acid isoforms, primarily C16 and a reduction in long chain isoforms, primarily C24. Nuclear targeting correlated with the ability of B subunit to induce apoptosis in these cells. Therefore Gb_3 isoform intracellular vesicular trafficking plays a major role in determining cell sensitivity to verotoxin.

PROTEIN KINASE C ACTIVATION IN T84 INTESTINAL EPITHELIAL CELLS INFECTED WITH SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI.*

V107/III

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STEC O157:H7 infection of cultured epithelial cells activates the phosphoinositide signal transduction cascade leading to increased levels of intracellular Ca^{2+} and inositol triphosphate. The objective of this study was to examine intracellular activation of protein kinase C (PKC) following STEC infection. Using a colorimetric assay, the proportion of PKC activity in the membrane fraction of T84 epithelial cells was increased following STEC-infection ($62 \pm 4\%$ of total) compared to both uninfected cells ($44 \pm 5\%$) and cells infected with the non-pathogenic *E. coli*, strain HB101 ($45 \pm 2\%$, $n = 5$; $p < 0.05$). STEC-induced PKC activity was comparable to that observed in T84 cells treated with phorbol 12-myristate 13-acetate ($71 \pm 4\%$), a known activator of PKC. Potential targets of STEC-activated PKC are now being examined for their possible role in the regulation of T84 tight junction permeability.

V108/III **DIVERGENT SIGNAL TRANSDUCTION RESPONSES TO INFECTION WITH SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC).**

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Cellular signals responsible for formation of attaching and effacing lesions by STEC in the infected host are undefined. STEC do not induce detectable tyrosine kinase responses in eukaryotic cells and are non-invasive. In the present study, phosphotyrosine proteins were detected under STEC and the bacteria were internalized when coincubated with an intimin-deficient EPEC, strain CVD206. The ability to rearrange phosphotyrosine proteins or internalize into epithelial cells did not occur following STEC coincubation with either another STEC or an EPEC *espB* mutant. Laboratory *E. coli*, strain JM101(pMH34/pSSS1C), which overproduces surface localized O157 intimin, coincubated with CVD206 also showed rearrangement of cytoskeletal proteins and detectable levels of phosphotyrosine proteins. In contrast, JM101(pMH34/pSSS1C) demonstrated rearrangement of only cytoskeletal proteins, but not phosphotyrosine proteins, when coincubated with STEC intimin-deficient strains (CL8KO1 and CL15). These findings indicate that STEC form adhesion pedestals by mechanisms that are distinct from those identified in attaching and effacing EPEC.

V120/III MUTATIONAL ANALYSIS OF THE VEROTOXIN-1 B-SUBUNIT EVIDENCE FOR THREE DISTINCT GB3 BINDING DOMAINS

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The Verotoxin-1 (VT1) B subunit binds to globotriaosylceramide (Gb3). Two putative carbohydrate [Galα(1-4)Gal] binding domains located adjacent to phenylalanine 30 (Sites I and III) have been identified by x-ray crystallographic and computer modelling studies. Co-crystallization studies of B subunit with a Pk trisaccharide analogue have demonstrated a third site (Site II) involving tryptophan 34. Amino acid substitutions were made for residues in all three sites to examine their roles in *in vitro* Gb3 binding and cytotoxicity. Mutations in all three sites caused significant reductions in *in vitro* Gb3 binding capacity while those at Sites I and III also caused reductions in binding affinity. Mutations affecting Sites I and III caused 4 to 7 log reductions in Vero cell cytotoxicity while Site II mutations caused only a 2 log reduction. Our results suggest that three potential receptor binding domains do exist on the VT1 B subunit. While Sites I and III appear to play a significant role in the cytotoxic action of the toxin, Site II appears to have minimal functional significance for cytotoxicity.

CONSTRUCTION OF A SERIES OF ISOGENIC, ACTIVE SITE, *slt I/slt II* MUTANTS OF A CLINICAL ISOLATE OF *E. COLI* O157:H7 **V121/III**

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EHEC are common contaminants of commercial food supply and cause a wide spectrum of disease in humans, including hemorrhagic colitis and hemolytic uremic syndrome. The role of the Shiga-like toxins in the pathogenesis of these clinical manifestations remains controversial. To address this question, we constucted deletion mutations of the *slt I* and *slt II* genes in a clinical isolate of *E. coli* O157:H7. 27 bp segments of the A subunits of the *slt I* and/or *slt II* toxin genes were deleted using inverse PCR, which removed the coding regions for the toxins' active sites at glutamic acid residue 167. The deleted fragments of the *slt I and II genes* were introduced into the wild type strain, 933, by homologous recombination using the positive selection suicide vector, pCVD 442. The resulting strains were compared to wild type for growth characteristics, production of toxin recognizable by polyclonal antisera raised to SLT I and II, and cytotoxicity to Vero cells in culture. DNA sequencing in the region of the toxins active site confirms the 27 bp inframe deletions. These series of toxin active site deletion mutations of *E. coli* O157:H7 will be invaluable in the evaluation of the comparative roles of the two toxins in pathogenesis and may be useful as a vaccine candidate.

ACID TOLERANCE OF *ESCHERICHIA COLI* : ASSOCIATION WITH PATHOTYPE AND SEROGROUP. **V122/III**

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The ability to tolerate low pH is associated with the survival of enterohemorrhagic *E. coli* (EHEC) in fermented foods and may be partly responsible for the low infectious dose of these bacteria. In this study we determine the ability of *E. coli* strains of various pathotypes and serogroups to tolerate pH 2.5 to 3.0. The results showed that EHEC and enteropathogenic *E. coli* (EPEC) collectively demonstrated levels of acid tolerance similar to each other, and that both EHEC and EPEC tolerated acid significantly better than enteroaggregative strains of *E. coli*. Regardless of pathotype, *E. coli* strains of serogroups O157 and O111 were more acid tolerant than those of serogroups O91 and O5. Serogroups O26 and O128 strains showed intermediate tolerance. These findings lend weight to the suggestion that acid tolerance is associated with virulence of EHEC and the ability of certain strains to infect hosts when a small number of bacteria are ingested.

V123/III VIRULENCE-ASSOCIATED CHARACTERISTICS OF VTEC STRAINS FROM PATIENTS WITH THE HEMOLYTIC UREMIC SYNDROME (HUS) AND HEMORRHAGIC COLITIS (HC)

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36 isolates of VTEC from serogroups O111 (14 strains), O157 (10), O26 (3), O48 (2) and O5, O6, O9, O113, O116, O130 and ONT (1 strain each), were examined for toxin production and adherence characteristics. 33 isolates produced enterohemolysin which correlated with carriage of the EHEC plasmid; 28 strains hybridised with a DNA probe for entero-aggregative heat-stable enterotoxin; 28 strains hybridised with a probe prepared from the *eae* gene. All of the eae-negative strains carried the EHEC plasmid. Most 0111 strains hemagglutinated erythrocytes from humans and pigs, but no strain agglutinated red cells from rats, guinea pigs, sheep, horses or chickens or adhered to intestinal brush borders from rabbits, calves or humans. In general VTEC strains adhered more strongly to Chinese hamster ovary cells than to HEp-2 or Int-407 cells, but there was no consistent pattern of adherence nor any relationship between the carriage of a particular virulence property and adhesion. The results indicate the heterogeneity of the in-vitro virulence-associated characteristics of VTEC strains associated with HUS and HC.

V131/III NEW VT2 VARIANT SEQUENCES IN NON-O157 VTEC ISOLATES

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By applying the PCR-RFLP VT2 variant B subunit identification scheme of Tyler et al. (JCM 91,29:1339) on 176 VT2-producing *E. coli* isolated from humans and meats, 52 of 109 non-O157 and none of 67 O157 strains harboured not typable VT2 genes (no amplification with VT2c & VT2d primers). Sequencing the genes of 4 selected strains revealed high homology with the recently published VT2/OX3a (EMBL X65949). New typing tests were designed to complete Tyler's scheme: PCR with VT2cw & v2 which is positive with the new variants but not with other VT2 genes and PCR with VT2az & v2 which is positive with all human VT2 genes, followed by restriction with *Pvu*II (cleaving only the new variants) and *Hae*III. Application of these tests confirmed the presence of VT2/OX3a related sequences in the other strains with not typable VT2 genes; most strains lacked 1 of the 2 *Hae*III restriction sites of VT2/OX3a. In conclusion sequences similar to VT2/OX3a (that shares several sequence variations with VTe and is probably less pathogenic) are frequent in non-O157 isolates, both from humans and from meats.

Effect of Sub-inhibitory Concentrations (SIC) of Sulfasalazine on Virulence of Verotoxigenic *Escherichia coli* (VTEC) **V135/III**

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Evidence from clinical studies suggests that the administration of antimicrobials to patients with verotoxigenic *E. coli* infection may be associated with an unfavorable clinical outcome. This is supported by in vitro studies which have shown an increase in verotoxin (VT) production when strains are exposed to SIC of some antimicrobials. Sulfasalazine has anti-bacterial activity and, as this agent is used in the management of ulcerative colitis- whose symptoms may overlap with hemorrhagic colitis- we investigated the effects of SIC of this compound on the production of VT1 and VT2 by clinical strains of VTEC using cell cytoxicity assays. In addition, the effects of sulfasalazine on the adherence of VTEC to T84 and Hep-2 cells was determined using quantitative and semi-quantitative methods including phase contrast microscopy. Additionally the presence of attaching effacing lesions was assessed using fluorescent-actin screening. Results will be discussed with reference to an on-going study which is examining the effects of an extended range of antimicrobials on adherence and VT production by VTEC.

EFFECTS OF VEROCYTOTOXIN ON THE NET ABSORPTIVE WATER FLUX IN HUMAN COLON *IN VITRO* **V138/III**

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The purpose of this study was to characterize the VT effects on the water and electrolyte transport across the human colon *in vitro*. We have used an experimental approach that allows the simultaneous recording of the net water flux (Jw), electrical potential different (PD) and short-circuit current (Isc). Colon specimens were obtained from chirurgically extirpated organs in patients with cancer. Inmediately after ablation, pieces of macroscopically non affected regions were washed and the muscle layer were dissected. Then the mucosa layer was mounted as a diaphragm between two Ussing chambers and bathed with identical Ringer solution in the presence of an hydrostatic transepithelial gradient of 13 cm of H_2O. In these conditions, a spontaneous absortive Jw of 0.19 ± 0.02 (10) $\mu l\ min^{-1}\ cm^{-2}$ was observed. When a crude VT2 preparation from *E. coli* C600 (933W) strain containing 16000 CD50 was added into the mucosal bath, Jw was inhibited 36% in the first 30 min. The Isc measured at the same time does not showed significant changes in the presence of VT2. These preliminar results shows that VT2 is able to inhibit the net absorptive water flux observed in human colon *in vitro*.

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V145/III SUBINHIBITORY CONCENTRATIONS OF ANTIBIOTICS INCREASE THE RELEASE OF SHIGA TOXIN FROM *E. COLI* O157:H7 IN VITRO

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The presence of subinhibitory concentrations of antibiotics (abx) appears to influence the release of Shiga toxin (Stx) by *E. coli* O157:H7. However, previous in vitro and human studies have shown conflicting results. In order to better evaluate this effect we used an enzyme immunoassay to measure the quantities of Stx in broth cultures of O157:H7 grown in the presence of either Ampicillin, Trimethoprim-Sulfa, Ciprofloxacin, Fosfomycin, or no abx, at 1/2 to 1/4 the MIC of each drug. Stx in the culture supernatants was markedly increased in the presence of abx, compared with the abx-free culture: Ampicillin: 10-14 fold; Trimethoprim-Sulfa: 47-72 fold; Ciprofloxacin: 31-52 fold; Fosfomycin: 13-20 fold. This difference was not accounted for by cell lysis, as determined by OD_{600}. There was little change in cell-associated Stx in the abx-containing versus abx-free cultures. If similar effects occur in vivo, increased release of free (and thus bioavailable) Stx in the intestinal lumen may alter the course of disease. Further studies are underway to explore this issue.

V150/III INDIVIDUAL VARIATION OF THE CONTENT OF Gb3, THE RECEPTOR FOR VEROTOXIN, IN HUMAN FETAL MEMBRANES

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Susceptibility to hemolytic uremic syndrome (HUS) after VTEC infection might depend on the content of Gb3 in tissues, but individual variation of Gb3 in tissues is not clear except that in erythrocytes. In this study, we analyzed contents and ratios of Gb3 and other neutral glycolipids in human fetal membranes collected from individuals just after the delivery. Amnion and chorion were obtained from 30 individuals and neutral glycolipids were analyzed by thin layer chromatography. Contents of CDH, Gb3 and Gb4 in amnion and chorion varied among individuals. The ratio of Gb3 in total neutral glycolipids of amnion was correlated with that of chorion (ρ=0.554, p=0.003), suggesting that the variation does not result from experimental errors. The ratios of CDH in both tissues were also correlated (ρ=0.457, p=0.014). These results show that CDH and Gb3 in fetal membranes varies among individuals. Therefore, Gb3 content in other tissues are potentially diverse and this might affect the susceptibility to HUS after VTEC infection.

CHARACTERIZATION OF THE LOCUS OF ENTEROCYTE EFFACEMENT OF E.COLI O157:H7. V159/III

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Enterohemorrhagic *E.coli* O157:H7 (EHEC) and enteropathogenic *E.coli* (EPEC) both exhibit the attaching/effacing (A/E) histopathology on intestinal epithelial cells. This phenotype is mediated by genes encoded on a 35kb pathogenicity island called the LEE for locus of enterocyte effacement. The LEE contains genes encoding intimin, secreted proteins, and a type III protein secretion system. In this study, we have characterized genes contained on the LEE of O157:H7. The EHEC LEE was shown to be homologous with the EPEC LEE over the entire 35kb region by the use of DNA probes. The complete EHEC LEE was cloned into a cosmid vector and it mediated weak A/E but was unable to mediate A/E or type III secretion at wildtype levels. Complementation of the EHEC LEE with subclones from the EPEC LEE, adhesins, or regulatory genes did not increase A/E or secretion. The function of EHEC LEE genes was also examined by chromosomal mutation of LEE ORFs, focussing on genes shown to be necessary for A/E in EPEC. So far, we have abolished A/E and type III secretion with mutations in ORF4 and ORF1, an HNS-transcriptional regulator homolog. These studies will help elucidate the intestinal aspects of EHEC colonization and virulence.

INSERTION OF THE LOCUS OF ENTEROCYTE EFFACEMENT IN EPEC AND EHEC STRAINS DIFFERS IN RELATION TO THE CLONAL LINEAGE OF THE STRAIN V161/III

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The locus of enterocyte effacement (LEE) confers the attaching and effacing histopathoogy on epithelial cells infected with enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) both *in vivo* and *in vitro*. We investigated the site of insertion of the LEE pathogenicity island in *E. coli* strains in relation to the evolution of housekeeping proteins in these strains. Using a PCR technique that specifically detects the insertion site of the LEE into the *selC* tRNA locus we investigated a total of 34 strains comprising representatives of 9 different clonal lineages. These clonal lineages are referred to as the DEC (diarrheagenic *E. coli*). The results indicate that the LEE insertion site varies according to the evolutionary DEC lineage, thereby indicating that the LEE has inserted at multiple sites during the evolution of these pathogens.

V162/III

Gb3/CD77 ON BOVINE PBMC: ACTIVATION MARKER AND/OR SHIGA TOXIN RECEPTOR?

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To investigate the expression and function of Gb3/CD77 on bovine immuno cells, bovine peripheral blood mononuclear cells (PBMC) were cultivated and co-incubated with mitogens (ConA, PHA-P, PWM, LPS) and purified Stx1, respectively. Cell morphology and membrane integrity, single PBMC subpopulations, and Gb3/CD77 were monitored by flow cytometry.
Freshly prepared PBMC did not express Gb3/CD77. However, during cultivation Gb3/CD77 was detected on all subpopulations investigated (BoCD4$^+$, BoCD8$^+$, BoCD21$^+$, WC1$^+$, and monocytes). The percentage of Gb3/CD77 expressing cells as well as the level of Gb3/CD77 expression on single cells were associated with the cells' grade of activation. Gb3/CD77 expression seemed to be a characteristic feature of distinct stages during the activation of bovine PBMC. Stx1 caused a marked reduction of Gb3/CD77 expression. Although this phenomenon was Stx1-specific, Stx1 was only partially bound by the cells. In conclusion, the vast majority of Gb3/CD77 on bovine PBMC is an activation marker, rather than a functional Stx receptor.

V166/III

ANALYSIS OF THE SHIGA TOXIN OPERONS FLANKING REGIONS IN *E. COLI* O157 AND NON O157 STRAINS

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Shiga toxin 1 (stx_1) and 2 (stx_2) genes are located in the genome of lambdoid phages in *E. coli* O157:H7 strains. In this study we compared the nucleotide sequences of an 11 kb region flanking the *stx* operons in *E. coli* O157, O26, O103 and O111 strains. In 6 of 8 strains possessing either stx_1 or stx_2 downstream of the P-gene, a region of ATGA-interlinked open reading frames (orf) like the *nin*-region of phage lambda was detected. Downstream of these *nin*-like genes we identified one orf which may code for an Q-like antiterminator. The stx_2 genes but not the stx_1 genes are in close association with an *ileX* tRNA gene found in *E. coli*. Downstream of stx_1 and stx_2 genes we found a number of conserved potential orfs of which one is homologous to the gene S of bacteriophage PA2. Our data show that Stx-converting phages in general follow the gene arrangement of phage lambda and indicate a potential for an extensive exchange with other lambdoid phages.

ATTACHING & EFFACING GENES ENCODING SECRETED SIGNALLING PROTEINS ARE ALSO REQUIRED FOR MODULATION OF HOST CELL ELECTROLYTE TRANSPORT

V181/III

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The pathophysiology of EPEC diarrhoea remains uncertain. In an Ussing chamber model using Caco-2 cells infected with EPEC, we previously demonstrated a partially chloride-dependent EPEC-induced stimulation of short-circuit current (Isc) which we suggested may contribute to the pathophysiology of EPEC diarrhoea *in vivo*. Several genes including *espA* *espB*, and *espD* encode secreted signalling proteins required for attaching & effacing (A/E) lesion formation. The aim of the present study was to investigate the role of these secreted proteins in EPEC-induced stimulation of Isc. In rapidly infected Caco-2 cell monolayers and concomitant with A/E lesion formation, wildtype EPEC strain E2348/69 induced a characteristic rapid increase in Isc. This response was absent when cells were infected with *espA*, *espB* and *espD* deletion mutant strains UMD872, UMD864 and UMD870 but was qualitatively restored when cells were infected with complimentary *espA* and *espB* plasmid transformant strains UMD872pMSD2(*espA*$^+$) and UMD864pMSD3(*espB*$^+$). This data suggests that signalling proteins required for A/E lesion formation are also required to induce alterations in host cell electrolyte transport.

CHARACTERIZATION OF A NOVEL PROTEIN SECRETED BY SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*.

V185/III

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Analysis of the supernatants of Shiga toxin-producing *E. coli* (STEC) revealed that at least four different polypeptides are efficiently exported in a temperature- and medium-dependent fashion. By N-terminal sequencing we identified proteins of 37 and 25 kDa as the STEC homologues of the EPEC proteins EspB and EspA. The N-terminus of a novel 104 kDa protein (p104) showed similarities to members of the IgA1 protease-like family, a finding that was confirmed by cloning and sequencing of the corresponding gene. Monoclonal antibodies were raised against p104 to investigate its presence in different STEC and EPEC strains and to analyze its secretion mechanism.

V193/III

FUNCTIONAL ANALYSIS OF THE ESPB GENES FROM ENTEROPATHOGENIC *ESCHERICHIA COLI* (EPEC), ENTEROHEMORRHAGIC *E. COLI* (EHEC), AND RDEC-1

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The attaching/effacing (A/E) lesion occurs with EPEC, EHEC, and rabbit RDEC-1 which possess the locus of enterocyte effacement (LEE). Tyrosine phosphorylation of host cells induced by the *espB* gene is known for EPEC but questioned in EHEC and unknown in other A/E strains. To compare *espB* function of EPEC, EHEC, and RDEC-1 we 1) cloned and sequenced *espB* from RDEC-1 (O15:H-), EHEC 85-170 (O157:H7), EHEC 6549 (O26:H11); 2) compared the sequences to EPEC E2348/69 (O127:H6) and EHEC EDL933 (O157:H7); 3) defined tyrosine phosphorylating ability of E2348/69, 85-170, and RDEC-1 on HEp-2 cells; 4) introduced a cloned *espB* of EPEC and RDEC-1 on pBluescript into UMD864 (*espB*-) and EHEC strain 85-170. Sequences of RDEC-1 and EHEC 6549 were identical. RDEC-1 (and 6549) and EHEC 85-170 differed by 22.9% and differed by 26.4% (RDEC-1) and 31.6% (EHEC) from EPEC. EHEC 85-170 and EDL933 differed at 3 bp. The central region of *espB* is highly polymorphic. Following 6 h incubation EPEC and RDEC-1 induced tyrosine phosphorylation detected by fluorescence. Minor fluorescence was seen by EHEC 85-170. Although introduction of the EPEC *espB* into UMD864 restored function seen by FAS indicating that the clone is functional, introduction of *espB* from EPEC or RDEC-1 into EHEC 85-170 did not increase its phosphorylating ability. These results confirm the relative failure of O157:H7 strains to induce tyrosine phosphorylation *in vitro* and the failure to produce levels of tyrosine phosphorylation similar to EPEC after introduction of EPEC *espB* suggests differences in transport of secreted proteins or adherence.

V202/III

ADHERENCE OF ESCHERICHIA COLI 0157:H7 TO EUKARYOTIC CELLS

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Adherence has been shown to be an important determinant of Escherichia coli 0157:H7 pathogenicity. The following data relates to the interaction between E.coli 0157:H7, cultured under different physiological conditions, and two eukaryotic cell lines - HeLa cells and human colonic epithelial cells. Quantitative, comparative adherence assays were performed and the maximum bacterial adherence was evident with exponential growth phase cells at pH6. Low iron concentrations decreased adherence while bacterial cells cultured under anaerobic conditions showed a marked increase in binding to HeLa cells. Polyacrylamide gel electrophoresis of outer membrane proteins demonstrated the expression and repression of defined proteins under these varied culture conditions. It can be concluded that E.coli 0157:H7, in response to specified cultural/environmental conditions synthesise specific adhesins which mediate binding to mucosal surfaces. In addition, ultrastructural studies of the adherence mechanism(s) has demonstrated the ability of E.coli 0157:H7 to invade selected human epithelial cell lines.

SHIGA-LIKE-TOXIN GENE OF Escherichia coli ISOLATED FROM DISEASED AND HEALTHY PIGLETS. V221/III

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DNA and biological probes specific for genes coding for Shiga-like-toxins (SLT-I and SLT-II) and for the enteromorrhagic factor (EHF), were used to examine fecal E. coli isolates from both diarrheic and healthy piglets.

No SLT-I positive hybridization was found. However some isolates from healthy as well as diseased animals possesed SLT-II genes. E. coli isolates from diseased piglets hybridize with the EHF DNA probe.

INTIMINS FROM ENTEROHEMORRHAGIC *ESCHERICHIA COLI* (EHEC) AND ENTEROPATHOGENIC *E. COLI* (EPEC) ARE FUNCTIONALLY HOMOLOGOUS V222/III

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The outer membrane protein intimin (Int) is necessary for the intimate attachment of both EHEC and EPEC to host cells. Int_{EHEC} and Int_{EPEC} show significant sequence homology, but differ greatly at their C-terminal, host cell binding domains. In order to study the binding properties of Int_{EHEC} and Int_{EPEC}, we constructed a fusion protein containing the C-terminal domain of Int_{EHEC} and compared it to an Int_{EPEC} C-terminal fusion protein, using immunofluoresecent microscopy and whole cell ELISA techniques. Int_{EHEC} and Int_{EPEC} fusion proteins bound to either EHEC- or EPEC-infected HeLa cells, but not to uninfected cells. Results from ELISA experiments demonstrate that Int_{EHEC} and Int_{EPEC} compete for binding to EHEC or EPEC infected HeLa cells, and bind with comparable affinity. These data suggest that Int_{EHEC} and Int_{EPEC} are functionally interchangeable.

V230/III

IDENTIFICATION OF A NOVEL FIMBRIAL ANTIGEN IN EHEC.

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The adherence factors of enterohemorrhagic *E. coli* (EHEC) have not been fully characterized and protective antigens have yet to be identified. In an effort to characterize novel fimbrial antigens, two prototype EHEC strains were chosen for fimbrial analysis: 933 (O157:H7) and 1639-78 (O26:H11). After growth on CFA agar, both strains displayed rigid fimbriae on their surfaces by transmission electron microscopy (TEM). Fimbrial proteins were purified by shearing and cesium chloride isopycnic ultracentrifugation. On SDS-PAGE, both organisms were found to display predominant protein bands of 21 kDa. The following N-terminal sequences were obtained: O157:H7: DD**GTITINGL**VTX(K)(T) and O26:H11: AX**GTITINGL**; each revealed significant homology to *Bordetella pertussis* and *E. coli* F17 fimbrial sequences. Antisera to the O157:H7 and O26:H11 fimbriae each reacted with the homologous 21 kDa proteins on Western blot and the homologous fimbrial structures on immunogold TEM. At dilutions of 1:2000 or greater, the absorbed sera only detected fimbriae of the homologous strain; at lower dilutions, antibodies from either fimbria reacted with both. Further experiments with additional EHEC strains indicated that the anti-fimbrial sera reacted with most isolates of the homologous EHEC serotype. One or both of the antisera reacted with 90% of EHEC,but only rarely with other *E. coli*. Plasmid-cured EHEC 933 still reacted with the antiserum, suggesting that the fimbriae were encoded by the EHEC chromosome. We hypothesize that EHEC express highly conserved surface fimbrial antigens which can be used as targets for rapid diagnostic reagents and as potential protective antigens in vaccine development.

V231/III

CONSTRUCTION OF REPORTER GENE FUSIONS TO STUDY THE INFLUENCE OF ENVIRONMENTAL FACTORS ON VERO-TOXIN EXPRESSION IN *ESCHERICHIA COLI* O157:H7.

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At present little is known about how environmental factors influence the expression of vero-toxins. Several bacterial virulence genes are known to be maximally expressed under conditions of environmental stress and this has lead us to speculate that such stresses may upregulate the expression of vero-toxin in *E. coli* O157:H7. We have constructed vero-toxin reporter gene fusions, allowing us to monitor the expression of vero-toxin at the colony and single cell level. The reporter genes used were the *E. coli lacZ*, and jellyfish *gfp*A genes. Fusions of the vero-toxin 2 A and reporter genes were achieved by a PCR splicing technique, and the fused DNA was subcloned into a vector containing a kanamycin resistance gene, the *B. subtilis sac*B gene, lethal when expressed in *E. coli* in the presence of sucrose, and a temperature sensitive origin of replication. A chromosomal gene fusion is created by allelic exchange in two stages, selecting for, 1) kanamycin resistance at the non-permissive temperature, and 2) ability to grow on medium containing sucrose and associated loss of kanamycin resistance.

CHARACTERIZATION OF SHIGA TOXIN-PRODUCING NON- O157 *ESCHERICHIA COLI* FROM THE UNITED STATES, 1983-1997 **V235/III**

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In the past 15 years, we have investigated clusters of Shiga toxin-producing *E. coli* (STEC) O104:H21, O111:NM and O121:H19. Among the 68 STEC isolates from sporadic infections, all with clinical information were from persons with diarrhea or hemolytic uremic syndrome. Thirty-four serotypes were isolated from sporadic cases, with O111:NM (11 isolates) and O26:H11(9 isolates) most common. Excluding epidemiologically-related isolates, 41% (30/72) of cluster and sporadic isolates were positive by PCR for only *stx1*, 35% (25/72) for only *stx2*, and 24% (17/72) for *stx1* and *stx2*. Sixty-five of the 72 were available for testing by PCR for *eaeA* and the EHEC plasmid; 68% (44/65) had both markers, 22% (14/65) had neither, 2% (1/65) had *eaeA* alone, and 9% (6/65) had the EHEC plasmid alone. Because a mucin-activatable form of Stx2 was recently reported for one of our strains lacking *eaeA* and *stx1* genes (O104:H21), we examined all the strains for an association between *eaeA* and *stx* genes. Twelve (60%) of 20 *eaeA*-negative strains lacked *stx1* compared with 11 (24%) of 45 *eaeA*-positive strains, ($p< 0.02$). Research is needed to determine if there is an association between the absence of *eaeA* and the production of activatable forms of Shiga toxins to better understand how these strains cause disease.

IDENTIFICATION OF SHIGA TOXIN PRODUCING *E. COLI* (STEC) FROM HUMAN STOOL BY COMBINED USE OF DIFFERENT SCREENING SYSTEMS **V30/IV**

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148 bacterial cultures grown from stool of human patients with supposed STEC infections were examined for STEC by the Verocell-toxicity (VT) test, an stx-specific PCR and the enterohemolysin (Ehly) agarplate assay. Thirtyfour (23.0%) of the cultures reacted positive in the stx-PCR, 64 (43.2%) were VT-positive and 50 (33.8%) yielded $Ehly^+$ *E. coli.* STEC could be isolated from 39 (26.4%) samples. All 39 samples were VT-test positive, but only 27 (69.2%) reacted in the stx-specific PCR. A negative stx-PCR result was obtained when low amounts of STEC were present in the stool culture. The VT-test was found to be more sensitive for detection of low amounts of STEC but reacted also positive with stool cultures containing other toxins than Stx. EHly-positives were found in 36 (92.3%) of the 39 STEC isolates. Our results are indicating that the combination of different screening methods provides the best chance for identification and isolation of STEC from stool. The Ehly-agarplate assay was suitable for isolation of low amounts of $Ehly^+$-STEC from dilutions of human stool cultures.

V35/IV THE ROLE OF LIPOPOLYSACCHARIDE AND SHIGA-LIKE TOXIN IN A MOUSE MODEL OF *ESCHERICHIA COLI* O157:H7 INFECTION

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The role of lipopolysaccharide (LPS) and Shiga-like toxin (SLT) in the pathogenesis of hemolytic uremic syndrome (HUS) was studied in a mouse model. Mice inoculated intragastrically with *E. coli* O157:H7 developed gastrointestinal, neurologic and systemic symptoms, necrotic foci in the colon, glomerular and tubular histopathology, and fragmented erythrocytes. LPS responder (C3H/HeN) mice developed a combination of neurological and systemic symptoms whereas LPS non-responder (C3H/HeJ) mice had a biphasic course of disease first developing systemic symptoms and later severe neurological symptoms. Mice inoculated with SLT-II-positive strains developed severe neurotoxic symptoms, a higher frequency of systemic symptoms and glomerular pathology compared to SLT-II-negative strains. Anti-SLT-II antibodies protected against these symptoms and pathology. These results demonstrate that this model could be used to study aspects of human HUS and that both LPS and SLT are important for disease development.

V49/IV MOLECULAR ANALYSIS OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* O111:H$^-$ PROTEINS WHICH REACT WITH CONVALESCENT HUS PATIENT SERA

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A recent outbreak of HUS in South Australia was caused by fermented sausage contaminated with STEC. The predominant STEC isolated from HUS patients belonged to serotype O111:H$^-$. We have performed Western blot analysis to assess the reactivity of five convalescent patient sera to O111:H$^-$ whole cells, untreated or treated with proteinase K. As expected, all five sera demonstrated a marked anti-LPS response, but several protein bands were also labelled. One convalescent serum was subsequently used to screen an O111:H$^-$ cosmid bank and two of nine hundred cosmid clones were found to be positive. Western blot analysis of these two clones identified three major immunoreactive protein bands of approximately 94, 70, and 50 kDa. An immune response to the three proteins was detectable in all five convalescent sera, but not in normal human serum. Preliminary studies have shown the 94 kDa protein is membrane-associated. Interestingly, it is also present in an enteropathogenic *E. coli* strain of serotype O111, but is not found in STEC strains belonging to other serotypes.

NEUROTOXICITY OF STX 2 AND PROTECTION BY ANTI STX2 ANTIBOBY IN RABBITS

V76/IV

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The brain lesions in rabbits given intravenous Shiga toxin 2 (Stx2) were noted at 24h in an area around the third ventricle (Infect. Immun. 1996. 64: 5053-5060). This result implied that Stx2 is present in the cerebrospinal fluid (CSF) despite toxin being administrated intravenously. We examined whether anti-Stx2 antibody injected intrathecally protects rabbits against brain damage. Eighty percent of the rabbits injected with Stx2 of 5.0 μg/kg, died within 8 days from brain damage. Rabbit anti-Stx2 sera were administrated into the CSF space through the cisterna magma. All the rabbits survived when they were given an intrathecal injection of the anti sera 2h before the intravenous injection of Stx2. Our results suggest that an intrathecal injection of anti-Stx2 antibody could be a therapy for acute encephalopathy by Stx2-producing *E. coli*.

EFFECT OF SHIGA TOXIN 2 ON AUTONOMIC CARDIOVASCULAR AND RESPIRATORY FUNCTIONS IN CONSCIOUS RABBITS

V77/IV

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In order to examine a possible cause of death after the Shiga toxin 2 (Stx2) administration, we monitored continuously and simultaneously the changes of cardiovascular and respiratory functions together with renal sympathetic nerve activity(RSNA) in conscious rabbits. All rabbits died about 45.5 hours in average after 20 μg/kg i.v. injection of Stx2. Arterial blood pressure, respiratory rate, PaO_2, $PaCO_2$ and RSNA were maintained at each normal level up to a few hours before death. Ataxia was observed on hindquarters at first, and then gradually extended to forequarters over ten hours before death. Thereafter, decrease in blood pressure and respiratory rate, and RSNA increase occurred almost simultaneously a few hours before death. These results suggest that the cause of the death might be dysfunction of the central nervous system, presumably due to prolonged effect of Stx2.

V80/IV

LIMPHOTOXIC EFFECT OF VEROCYTOTOXIN PRODUCING *Escherichia coli* IN RABBIT CAECUM.

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An experimental model comprising in situ inoculation of VT-producing *Escherichia coli* strains in a surgically isolated caecum segment of rabbits was developed. Cultures of strains 933, 933J, 933W and K12 were tested; rabbits were clinically evaluated until they died or euthanasia performed (six weeks after surgery); blood and urine samples were taken for complete hemogram and urea and creatinine evaluation. Histopathological examination of the caecum segment revealed a severe lymphocyte depletion, destruction of enterocytes, interstitial edema and hemorrhage. Lesions were more severe in rabbits inoculated with strain 933; milder lesions were observed after inoculation with strain 933J

V81/IV

A RABBIT MODEL FOR HAEMOLYTIC UREMIC SYNDROME.

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An experimental model consisting of in situ inoculation of VT-producing *Escherichia coli* strains into a surgically isolated caecum segment of rabbits was developed. Rabbits inoculated with strains 933 or 933W developed hemorrhagic diarrhoea, anemia and renal failure over a period of 2 to 5 days post inoculation; milder lesions were observed in rabbits inoculated with strain 933J; in contrast rabbits inoculated with strain K12 remained healthy over a period of six weeks after inoculation. The animals developed increase of blood nitrogen urea, creatinine and haemoglobin in serum and decrease of seric free haptoglobin. This rabbit model reproduced the illness and the hystological alterations described in Haemolytic Uremic Syndrome.

A LABORATORY MODEL OF HAEMOLYTIC URAEMIC SYNDROME (HUS). V83/IV

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An animal model, essential to explore the pathogenic pathways of HUS, is constrained by the species restriction of Gb3. We have circumvented this problem by using the plant toxin ricin which has identical enzymatic activity on ribosomes.
Rats given intravenous ricin or LPS (E. coli O111:B4) separately had no thrombotic microangiopathy at 8h. Simultaneous treatment caused typical glomerular thrombotic microangiopathy, replicating the histology seen in humans. This is an interogatable animal model for human HUS.

SHIGA TOXIN 1 (STX1) INTERACTION WITH HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS: CYTOKINES AS SENSITIZING AGENTS. V101/IV

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Neurologic abnormalities are among the most serious extra-intestinal sequelae of infection with Stx-producing bacteria. Earlier studies suggested that Stxs do not directly damage neuronal cells; rather vascular endothelial cells may be the primary targets of Stx-mediated cytotoxicity. We show here that human brain microvascular endothelial cells (HBMEC) cultured *in vitro* were relatively resistant to purified Stx-1 (CD_{50} ~10 µg/ml or 10^4 Vero CD_{50}s). Pretreatment of HBMEC with TNF-α, IL-1β, butyric acid or a cAMP analogue resulted in a 10^3- to 10^5-fold decrease in CD_{50}s, and a 2- to 3-fold increase in the binding of FITC-labeled Stx1 to HBMEC. Interestingly, LPS pretreatment of HBMEC did not alter toxin sensitivity or binding. These data suggest that the Stx1-mediated host response may participate in the development of neurologic complications. Studies to elucidate and quantitate the glycolipids interacting with Stx1 are in progress. (Supported by USPHS grant AI34530).

V102/IV **SHIGA TOXIN 1 (STX1) ACTIVATES TNF-α GENE TRANSCRIPTION AND TRIGGERS NUCLEAR TRANSLOCATION OF THE TRANSCRIPTIONAL ACTIVATORS NF-κB AND AP-1.**

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Dysentery caused by Stx-producing bacteria is linked with development of acute renal failure and neurologic abnormalities. The pathologic hallmarks of post-diarrheal sequelae are thrombotic vascular lesions. *In vitro* studies using human vascular endothelial cells (HVEC) demonstrated minimal Stx cytopathic effects, unless the target cells were also incubated with the cytokines TNF-α or IL-1β. TNF-α increases toxin receptor expression by HVEC *in vitro*. We show here that purified Stx1 or LPS induces TNF secretion by a human monocytic cell line in a dose- and time-dependent manner. Treatment of cells with Stx1+LPS resulted in augmented TNF production. Northern blot analyses showed that Stx1-mediated TNF-α induction is mediated, at least in part, at the transcriptional level. Increased levels of TNF-α mRNA were preceded by the nuclear translocation of the transcriptional activators NF-κB and AP-1. Collectively, these data suggest that Stxs possess cellular signaling capabilities sufficient to induce the synthesis of the cytokines that are instrumental in target cell sensitization and the development of vascular lesions. (Supported by USPHS grant AI-34530).

V104/IV **A PRIMATE MODEL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* INFECTION**

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Twenty-two adult Macacca radiata were infected with *Escherichia coli* O157:H7 strain 84-01. Diarrhea occurred for upto 5 days post-infection in 17 monkeys. O157 were isolated from about 6 hours upto 12 days post-infection in 17 monkeys. Acute colitis with submucosal congestion and attaching-effacing (A/E) lesions were present from 6 hours to 9 days, mainly in the caecum, ascending and mid-colon. Ultrastructurally, A/E lesions specifically targeted the intercellular junctions with marked epithelial degeneration. Secondary bacterial infection followed O157 infection and subsequent barrier loss. This primate model parallels early stages of disease produced by *E. coli* O157:H7.

SLT I AND II DAMAGE MICROVASCULATURE IN THE GI TRACT V106/IV

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LD50 dose of SLT I and II was parenterally administered to twelve 4-6 week old Swiss albino mice. Ultrastructural examination of the hepatogastrointestinal vasculature showed damage to endothelial cells with swelling, cytoplasmic rarefaction, dilatation of endoplasmic reticulum, clumping of nuclear chromatin and focal disruption of cell membrane. In addition there was platelet aggregation, degranulation and adherence to damaged endothelium, maximally affecting the caecum. Capillaries and venules were more severely damaged than arterioles. Damage to microvascular endothelium could be a contributory factor in producing diarrhea in EHEC infection.

ADULT RABBIT AS AN ANIMAL MODEL FOR THE HEMOLYTIC UREMIC SYNDROME V109/IV

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Hemolytic uremic syndrome (HUS) is defined as a triad of acute renal failure, microangiopathic hemolytic anemia and thrombocytopenia. In this study we created Thiry-Vella loops in the small intestine of 12 rabbits. A 1 mL volume of a broth culture (6 x 10^8 CFU/mL) of Shiga-like toxin producing *Escherichia coli* (SLTEC) strains 933, 933J, 933W, H30 and negative control strain *E. coli* K-12 was inoculated into the isolated intestinal segments. The rabbits were observed and evaluated clinically over a 5-week period, during which blood urea, creatinine and serum haptoglobin were measured every 72 hours. The animals were euthanized at the end of the 5 weeks. Animals inoculated with SLTEC strains showed elevation of blood urea and creatinine. Histopathological examination showed obstruction of renal microvessels and glomerular and tubular necrosis in the kidneys, edema in the brain, erythrocyte fragmentation in the liver, and congestion in the spleen. Strain 933 elicited the most severe changes but all SLTEC induced changes similar to those seen in HUS. This animal model demonstrated that intact toxin passes from the intestine into the circulation.

V115/IV **INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1ra) PROTECTS AGAINST TISSUE INJURY IN AN ANIMAL MODEL OF HEMORRHAGIC COLITIS.**

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E.coli strain RDEC-H19A is an attaching/effacing (A/E) rabbit pathogen which secretes high levels of SLT-1. This strain induces intestinal disease in rabbits resembling human hemorrhagic colitis. Since IL-1, in vitro, upregulates endothelial receptors for SLT-1, inhibition of IL-1 activity, by administration of IL-1 receptor antagonist (IL-1ra) may attenuate the inflammatory response to enterohemorrhagic *E.coli* (EHEC) infection. To determine if IL-1ra could prevent tissue injury due to SLT-1 producing *E. coli*, we administered IL-1ra systemically to rabbits; introduced RDEC-H19A into ligated small bowel loops; confirmed colonization and toxin expression; measured fluid accumulation; and graded tissue injury by histo-pathological analysis. Intestinal loops in untreated rabbits injected with H19A were grossly edematous(0.3 ml/cm of fluid). In contrast, 10/12 intestinal loops in IL-1ra treated animals appeared grossly normal while 2 affected loops contained only 0.1ml/cm of fluid. Submucosal edema in untreated animals (241 $\pm$ 131um in H19A loops) was markedly decreased by IL-1ra treatment (7.5 $\pm$ 20um, $p<0.03$). Crypt/villus ratio (4.5$\pm$0.41um PBS loops) was decreased by H19A in untreated animals (2.2$\pm$0.3um, $p<0.03$) but maintained by IL-1ra (3.9$\pm$0.4um). PMN infiltration (13.2$\pm$4.8 PMN/villus H19A loops) was limited by IL-1ra (2.4$\pm$3.6, $p<0.01$). Bacterial mucosal adherence was unchanged by IL-1ra. IL-1ra markedly protected intestinal loops challenged with an SLT-1 producing, A/E strain of *E. coli* against edema, inflammation, and injury.

V125/IV COMPETITIVE COLONIZATION OF SHEEP BY DIFFERENT PATHOTYPES OF *ESCHERICHIA COLI*

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We developed a sheep model of competitive colonization to test whether enterohemmoragic *Escherichia coli* (EHEC) colonize the intestinal tract of ruminants more effectively than other pathotypes of *E. coli*. Six strains, representing 4 pathotypes, were selected: 2 enterotoxigenic (ETEC), 2 EHEC, 1 enteropathogenic and 1 nonpathogenic. The strains were differentiated from each other and from the resident flora by antibiotic resistance and biotyping. Two sheep were simultaneously inoculated with all 6 strains (10^{10} CFU/strain) and fecal shedding of individual strains was followed. All 6 strains were detected by 4 days post inoculation (PI) at 10^2 to 10^5 CFU/g of feces. Two weeks PI, all 6 strains were 10^2 CFU/g or less. Two months PI the 2 EHEC strains were detected only by enrichment culture (<50 CFU/g) in both animals, 1 ETEC strain was detected in 1 animal (<50 CFU/g) and the other strains were not recovered. Our results suggest that EHEC colonize ruminants better than other pathotypes of *E. coli*.

AN ENZYME-LINKED IMMUNOSORBANT ASSAY TO DETECT PATIENT ANTIBODIES TO SECRETED PROTEINS OF *ESCHERICHIA COLI* O157:H7. **V160/IV**

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We recently reported that *Escherichia coli* O157:H7 secretes novel proteins that engender a strong immune response in patients infected with the organism (Infec. Immun. 64:4826;1996). Two of these proteins EspA (24 kDa) and EspB (37 kDa) are exported by a type III secretion system. The genes encoding EspA , EspB, and the type III secretion system, are contained on a 35 kb pathogenicity island called the LEE (locus of enterocyte effacement). A 100 kDa protein, not encoded for within the LEE, is also secreted extracellularly but not via the type III pathway. Western blot analysis of the polypeptides secreted by *E. coli* O157:H7 has shown that they are recognized by rabbit antiserum raised against the proteins secreted from enteropathogenic *E. coli* and by human sera from patients infected with *E. coli* O157:H7 strains. We have developed an ELISA to screen human sera for the presence of antibodies to the *E. coli* O157:H7 secreted proteins. Initial experiments using a whole secreted protein preparation as the antigen revealed that the 100 kDa protein reacted with potentially negative sera. We have cloned the *espB* gene from an *E. coli* O157:H7 strain into a fusion system that enables high levels of protein expression and subsequent purification of the cloned gene product without the fusion tag. The use of purified EspB protein in this ELISA offers a useful method for immunodiagnostics and seroepidemiology.

RABBITS IMMUNIZED WITH A VEROCYTOTOXIN 2 (VT2) TOXOID ARE CROSS-PROTECTED AGAINST CHALLENGE BY INTRAVENOUS VT1. **V163/IV**

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Verocytotoxin 1 (VT 1) and VT2 are neutralized by homologous but not by heterologous antisera in-vitro. Similarly, in-vivo, rabbits immunized with VT1 toxoid or Shiga toxoid are protected against intravenous challenge with homologous toxin, but whether they are also cross-protected against systemic challenge with a heterologous VT has yet to be established. The objective of this study was thus to determine whether rabbits immunized with a VT2 toxoid were cross-protected against intravenous challenge with VT1. Specific pathogen-free New Zealand White rabbits (~ 2kg) were immunized by subcutaneous injection with 50 µg of VT2 toxoid mixed with equal volume of Freund's complete adjuvant. (FCA) and boosted with 70 µg of VT2 toxoid in FICA in four sequential weekly intervals. Immunized animals developed neutralizing antibody titers ranging from 1:16,000 to 1:32,000 against VT2 but titers of < 1:2 against VT1. Two groups of three immunized rabbits and non-immunized rabbits were challenged with 10 and 50 LD_{50}s of VT1 respectively and observed for symptoms. All unimmunized animals developed evidence of hemorrhagic diarrhea, all but one, developed limb weakness, and two animals became tetraplegic. These animals were sacrificed in a humane manner. All immunized animals, however, remained symptom-free over an observation period of three and a half weeks. In parallel experiments, protection of VT2-immunized animals against VT1 challenge correlated with the lack of uptake of both ^{125}I-VT1 and ^{125}I-VT2 by target tissues of immunized animals but not by those of unimmunized animals. Our findings indicate that immunization with VT2 toxoid provides protection against challenge by homologous and heterologous toxins, and this has significant implications for the design of vaccine strategies in humans.

V164/IV LOCALIZATION OF INTRAVENOUSLY ADMINISTERED VEROCYTOTOXINS (SHIGA-TOXINS) 1 AND 2 IN RABBITS IMMUNIZED AGAINST HOMOLOGOUS AND HETEROLOGOUS TOXINS AND TOXIN SUBUNITS. M. Bielaszewska, I. Clarke, M. A. Karmali*, and M. Petric. Division of Microbiology, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, University of Toronto, Ontario, Canada.

The pathological effects of Verocytotoxin 1 (VT1) in the CNS and the GI tract of rabbits correlate with the uptake of ^{125}I-labeled VT1 by the same tissues. In animals immunized with VT1 toxoid, uptake of ^{125}I-VT1 by target tissues is inhibited and labeled toxin is cleared by the liver and spleen. The uptake of ^{125}I-labeled VT1 in immunized and non-immunized animals thus provides a convenient approach for studying immunity to systemic toxin. The objective of this study was to determine the uptake of ^{125}I-labeled VT1 and VT2 in rabbits immunized against homologous and heterologous toxins and toxin subunits. Groups of rabbits were immunized with VT1 toxoid, VT2 toxoid, or with the A or B subunits of each toxin, and challenged with intravenous ^{125}I-VT1 or ^{125}I-VT2. After 2 hours the animals were sacrificed, and selected tissues were analyzed for uptake of radiolabelled toxin. It was found that animals immunized with VT1 toxoid or with VT2 toxoid were protected from target tissue uptake of labeled homologous toxin as well as the labeled heterologous toxin, with the highest uptake of labeled toxins occurring in the liver and spleen. Similarly rabbits immunized by either the VT1A subunit or the VT2A subunit were protected from target tissue uptake of both the homologous and heterologous ^{125}I-labeled holotoxins. In contrast, in animals immunized with the toxin B subunits, protection extended only against challenge by the homologous toxin. These results provide evidence of VT1 and VT2 cross-neutralization in-vivo in the rabbit model and indicate that the in-vivo cross-neutralization is a function of antibodies directed to the VT A subunits. This suggests that the VT1A or VT2 A may be suitable immunogens for immunizing humans against systemic VT-mediated disease.

V167/IV VEROCYTOTOXIN PRODUCING *Escherichia coli* STRAINS ISOLATED IN MEXICO. DURING AN OUTBREAK OF BLOODY DIARRHOEA OF CALFS.

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In this study we are reporting clinical and actiologycal results about an outbreak of bloody diarrhea in calfs of 1 to 10 days old. Selective culture mediums and specific O and H antiserum were used to identified the ***E. coli*** strains. The verocytotoxic activity was determined in vero-cells cultures and in a rabbit model developed in our laboratory. The clinical features were related with haemorrhagic colitis with lymphocyte infiltration, and edema of the submucose in the digestive tract. Alterations of central nervous system, and death of some calfs also were observed. ***E. coli*** strains of serotypes O4:H16, O33:H$^-$, O114:H4, O157:H$^-$, O166:H15 and O167:H10 were identified, all the strains were cytotoxin producers. The rabbit model reproduced the illness and the hystological alterations described in Uremic Haemolytic Syndrome. Rotavirus were observed in the faecal samples without significative impute risk of infection associated to the illness ($p< 0.05$). In Mexico EHEC strains of serotypes different to O157:H7 are associated with haemorrhagic colitis in calfs.

SERUM ANTIBODIES TO *Escherichia coli* O157 LIPOPOLYSACCHARIDE IN MEXICAN ADULTS. **V168/IV**

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In Mexico there has not been found any illness in humans, associated to *Escherichia coli* O157:H7 infection. In this study we show results related to the serological cross-reaction between, *E. coli* O157 and other *E. coli* serogroups. The antibodies response against O157 lipopolysaccharide (LPS) is also reported, from serum samples obtained from healthy adults. Plate microagglutination test with specific rabbit antiserum obtained against the 173 *E. coli* somatic (O) antigens, was used to analized the LPS cross reactivity in 114 *E. coli* O157 strains.The presence of antibodies against O157 LPS was determined in 100 serum samples, by plate microagglutination and ELISA test. Cytotoxic activity of the O157 strains was measured with the Vero cells assay. O157 LPS shown cross reactivity against O7 (67%) and O116 (81%) LPS. The human serum response against O157 LPS was positive in 37% of the samples, and in 26% y 22% against O7 and O116 LPS. Culture supernatants from O157:H7 *E. coli* strains isolated in different countries, and O157 strains with different flagellar antigen isolated in Mexico were cytotoxic to Vero cells cultures. *E. coli* O157:H7 is a pathogen not associated to illness in Mexico; the presence of antibodies against O157 LPS in the serum is likely related to inespecific immune response associated to antigenic cross reactivity.

CHARACTERIZATION OF THE PRIMATE (BABOON) RESPONSES TO SHIGA-LIKE TOXIN. **V176/IV**

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The response of 10 baboons to IV infusion of Shiga-like toxin-I (SLT-I) varied from acute renal failure and hyperkalemia (Group 1, N=4) to classical hemolytic uremic syndrome (HUS) with renal failure thrombocytopenia, schistocytosis, anemia, and melena (Group 2, N=3) to renal failure with a mixed response (Group 3, N=2). Group 1 received 2.0 ug/kg of SLT-1. Light and electron microscopy showed organelle disintegration and necrosis of proximal tubular epithelium, and of mucosal epithelium at the tips of the microvilli of the intestine both of which bear Gb3 receptors. These changes also were accompanied by swelling of podocytes and retraction of endothelial cells of renal glomerular capillaries. The renal changes were multifocal. The intestinal changes were accompanied by minimal to extensive mucosal and submucosal hemorrhage. Electron microscopic images of brain cortex and cerebellum showed diffuse uniform unraveling of myelin sheaths with occasional disintegration of neuronal cell bodies. Gb3 receptors were not present in these CNS tissues.

Groups 2 and 3 received 0.05 to 0.2 ug/kg of SLT-1. Light and/or electron microscopy showed microvascular fibrin deposition and thrombosis of renal glomerular and peritabular capillaries in Group 2 in conjunction with systemic markers of HUS, while Group 3 showed a mixed picture. All three groups exhibited renal shut down and died in 62 hours or less. All these groups produced urine which was positive for tumor necrosis factor and interleukin-6 while neither of these cytokines was detectable (≤ 5 pg/ml) in the general circulation. We concluded that depending on the dose, SLT-1 can injure directly via its toxic effects and indirectly via host inflammatory mediators.

V188/IV

TOXIN ANTIBODIES IN THE SERA OF CHILDREN WITH *SHIGELLA*-ASSOCIATED HEMOLYTIC UREMIC SYNDROME

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Antibodies to Shiga-like toxin-1 (IgA, IgG and IgM) were measured in the sera of 54 children, aged 12-60 months, with *Shigella dysenteriae* 1 infection. Of the 54 children, 18 had HUS. Children with HUS had lower anti-toxin IgM titers than those without HUS ($p=0.008$). However, as multiple regression analysis revealed that the duration of diarrhea prior to admission influenced the antibody response, children were divided into short (3-5 days), medium (6-9 days) and long (>9 days) diarrhea duration subgroups. In the short diarrhea duration subgroup, children with HUS had higher anti-toxin IgA titers than children without HUS ($p=0.028$). In the medium and long diarrhea duration subgroups, IgM titers were lower in children with HUS than in those without HUS ($p=0.008$ and 0.020, respectively). These findings suggest that HUS occurs in children with a secondary infection and that anti-toxin IgA may not be protective against the development of HUS.

V189/IV

ADHESION OF NEUTROPHILS TO CEREBRAL ENDOTHELIAL CELLS INDUCED BY VEROTOXIGENIC *ESCHERICHIA COLI* (VTEC)

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Neutrophil adhesion and injury to cerebral endothelial cells may be important in the pathophysiology of the encephalopathy of hemolytic uremic syndrome. We hypothesized that the adhesion of neutrophils to cerebral endothelial cells would increase during the development of the cerebral microangiopathy induced by VTEC in mice. Mice were anesthetized at 24, 48 and 72 hours following intra peritoneal injection of crude extracts of VTEC and following no injection (controls). The adhesion of leukocytes to endothelial cells in pial venules was recorded in real-time using fluorescent intravital microscopy through a cranial window following intravenous injection of the nuclear dye, acridine orange. There was a four-fold rise in the mean number of leukocytes rolling and adhering in pial venules at 48 hours following injection of VTEC compared to controls. In conclusion, the adhesion of leukocytes to endothelial cells in pial venules increases following injection of crude extracts of VTEC in mice.

PLASMA AND STOOL CYTOKINES AND CYTOKINE ANTAGONISTS, PLASMA ENDOTOXIN, AND STOOL SHIGA TOXIN IN PATIENTS WITH *S. DYSENTERIAE* TYPE 1-ASSOCIATED HUS

V195/IV

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We measured on admission, hospital days 3 & 5, and post-discharge plasma concentrations of endotoxin (ETX), tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), granulocyte colony stimulating factor (G-CSF) granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-1 receptor antagonist (IL-1RA) tumor necrosis factor-binding protein (TNF-BP), and stool concentrations of TNF and Shiga toxin (STX) in 26 patients (pts) with *S. dysenteriae* 1 (SD1) infection and HUS, 36 pts with SD1 infection and leukemoid reaction, 65 pts with SD1 infection without systemic complications, 30 pts with *Shigella* infections other than SD1, and 103 pts with watery diarrhea. ETX was measured by chromogenic-limulus lysate assay. G-CSF and STX by ELISA; all others by RIA. All four *Shigella* groups had higher median peak ETX concentrations than did pts with watery diarrhea, with the highest concentrations being in pts with HUS (3.93 IU vs 0.21 in pts with watery diarrhea). HUS pts also had significantly higher median peak plasma concentrations of TNF, GM-CSF, IL-6, IL-RA, TNF-BP and higher stool concentrations of TNF than the other 4 groups. The 3 SD1 groups more commonly had SXT detectable in stool. In summary, HUS pts had higher endotoxin concentration, and a more marked inflammatory and counter-inflammatory response, than *Shigella* pts without HUS.

ANTIBODY RESPONSES TO VEROTOXIN 2 IN PATIENTS WITH ENTEROPATHIC HEMOLYTIC UREMIC SYNDROME (HUS).

V200/IV

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The presence of non-specific anti-VT2 neutralizing factors in serum has restricted understanding of the nature and frequency of antibodies to VT2 in patients with Verotoxin-producing *Escherichia coli* (VTEC) infection. To overcome this, we have successfully established a Western blotting method to detect IgG antibodies to VT2. Briefly, VT2 was purified from *E. coli* strain R82pJES 120DH5alpha (provided by Dr. J. E. Samuel) using sequential column chromatography (hydroxylapatite, chromatofocussing, and cibachron blue), and resolved into its A and B subunits by polyacrylamide gel electrophoresis. The protein bands were transferred by western blotting onto PVDF membranes (BIO-RAD), blocked for non-specific binding with Tris buffer containing 5% skim milk and 10% goat serum and reacted with test sera diluted 1:100. Bound immunoglobulin was detected using HRP-conjugated goat anti-human IgG (H + L) (BIO-RAD). The antigen-antibody system was developed using a chemilluminescent detection system (ICL; Amersham, UK) . We investigated sera from 94 patients with HUS and from 100 healthy age-matched controls. Overall, 66/94 (70%) of patients with HUS were positive for anti-VT2 IgG in contrast to 11% of controls. We conclude i) that Western blotting is an effective method for detecting antibodies to VT2, and ii) that patients with HUS frequently develop antibodies to VT2. Further work is needed to correlate the frequency of both anti-VT1 and anti-VT2 with the toxin genotypes in the infecting VTEC strain.

V217/IV EVIDENCE OF BOUND VEROTOXIN 1 TO THE TUBULAR CELLS OF AN AUTOPSY KIDNEY FROM A VTEC O157:H7 ($VT1^+$, $VT2^+$)-ASSOCIATED HUS PATIENT

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Autopsy samples of a 1y9m aged girl who died 25 days after the onset of VTEC O157 infection were analyzed. Ulcerative and granulomatous degeneration in the colon, massive ballooning and fatty degeneration of hepatocytes, interstitial pneumonia and focal hemorrhage in the lung, focal necrosis and degeneration in the kidney were seen. Further, the frozen section of the kidney was examined whether verotoxins were present or not. Mouse MAb-based immunostaining demonstrated the evidence that VT1 but not VT2 retained on the tubular cells of the kidney of the patient. Specific affinity of verotoxins to distal tubules was demonstrated using a frozen section of a kidney from a non-VTEC patient.

V19/V LACK OF EFFECT OF LOOP DIURETICS ON VEROTOXIN BINDING AND INTERNALIZATION IN VERO CELLS

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Clinical evidence supported the idea that treatment of active verotoxin induced HUS with a loop diuretic may decrease the necessity for dialysis treatment. The exact mechanism as to how these drugs may affect verotoxin cytotoxicity is unknown. Previous work has demonstrated that the entry of Shiga toxin into Vero cells appears to be exquisitely sensitive to the ionic composition of the intracellular and extracellular milieu. Since loop diuretics directly impair Na-K-Cl co-transport and thus may impact cytosolic and extracellular ionic concentrations, we studied the effects of two different loop diuretics, furosemide and bumetanide, on the degree of attachment and subsequent internalization of verotoxin to Vero cells. Fluoresceinated verotoxin B subunit was used to quantitate fluorescence measurements after one-half hour binding at 4°C and after 2 hours internalization at 37°C. There was no significant change in the binding or internalization of the B subunit in Vero cells in the presence of loop diuretics. This study does not support a direct role of loop diuretics in preventing cellular intoxication by verotoxin.

VEROCYTOTOXIN-2 (VT-2) INDUCES INCREASE OF CYTOSOLIC CALCIUM IN HUMAN NON-ADHERENT MONOCYTES (MO). V27/V

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In vitro the binding of VT to MO is followed by the production of cytokines. In the present study we analyzed whether calcium is involved in this pathway. Blood was collected from healthy male donors and MO were purified by apheresis followed by Percoll gradient and counterflow centrifugation. Purity of MO was at least 95%. The effect of VT-2 on intracellular Ca^{++} ($[Ca^{2+}]_i$) in MO (2.10^6/assay) was determined by the FURA-2AM method. MCP-1, known to increase $[Ca^{2+}]_i$ in MO, was used as positive control. VT-2 (10 nM) caused a rise in $[Ca^{2+}]_i$ in MO. The β-subunit alone did not induce any $[Ca^{2+}]_i$ response. When chelating extracellular Ca^{++} by EGTA (10 mM) it was still possible to induce an increase in $[Ca^{2+}]_i$ by VT-2. By combining EGTA and BAPTA-AM (15 μM) we did not observe any change in $[Ca^{2+}]_i$ upon VT-2 incubation. Preincubation of MO with genistein (50 μM) completely blocked the VT-2 effect on $[Ca^{2+}]_i$. In conclusion: 1.) In MO intracellular Ca^{++} stores play an important role in VT-2 induced $[Ca^{2+}]_i$ increase. 2.) The β-subunit alone did not result in a $[Ca^{2+}]_i$ response. 3.) Tyrosine kinase seems to be involved in the signal transduction pathway of VT-2 resulting in $[Ca^{2+}]_i$ release.

POSSIBLE CYTOKINE-MEDIATED AUTOCRINE AND PARACRINE REGULATION OF SLT-1 CYTOTOXICITY IN HUMAN GLOMERULAR AND TUBULAR CELLS. V56/V

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SLT may selectively increase renal cytokines which may, in turn, augment SLT-induced renal cell toxicity. To begin to examine this, we measured SLT-1 (and LPS for comparison) effects on IL-1, IL-6 and TNF production by cultured human glomerular endothelial (GEN), mesangial (MC), and proximal tubular (PT) cells. Cytokine modulation of SLT toxicity on these cells was also evaluated. In PT and GEN, but not MC, SLT increased TNF, IL1, and IL-6 production (protein and/or mRNA levels). In contrast, LPS increased cytokine production by MC and GEN, but not PT. Pre-incubation with LPS or IL-1 increased PT and GEN sensitivity to SLT, while TNF increased MC and GEN sensitivity to SLT. IL-6 did not alter SLT responsiveness in any cell type. These studies suggest that SLT and LPS together can induce release of cytokines by human glomerular and tubular cells which can, in turn, increase SLT toxicity on these cells. If this autocrine/paracrine system functions *in vivo*, it may partially explain unique renal cell damage in HUS.

V57/V VEROTOXIN-2 (VT-2) INDUCES APOPTOSIS IN HUMAN UMBILICAL VEIN- AND GLOMERULAR MICROVASCULAR ENDOTHELIAL CELLS.

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The pathogenesis of the HUS is hallmarked by endothelial damage of glomeruli/arterioles of the kidney. VT toxicity in human umbilical vein (HUVEC)- and glomerular microvascular endothelial cells (GMVEC) requires additional stimuli (TNF-α) and is related to inhibition of overall protein synthesis. VT is known to induce apoptosis in Vero- and Burkitt's lymphoma cells. The apoptosis inducing effect of VT in HUVEC, GMVEC and foreskin MVEC was subject of the present study. Apoptosis was investigated by flow cytometry (using propidium iodide (PI) and annexin V-FITC (A)), nuclear staining of cell monolayers (by Hoechst, PI and A) and DNA analysis. In VT-2 (10 nM) exposed, non-stimulated HUVEC and GMVEC no significant numbers of apoptotic cells were observed by flow cytometry. However in non-stimulated MVEC VT-2 exposure (10 nM) for 4 h and 24 h resulted in 13,1% and 34,7% apoptotic cells, respectively. The percentage of apoptotic cells in TNF-α prestimulated HUVEC and GMVEC (10 nM VT-2; 4-6 hr) was 17,1% and 14,1%, respectively. Apoptosis was confirmed by nuclear staining of cell monolayers and by DNA fragmentation analyses, showing characteristic DNA "ladder" patterns. In conclusion, VT-2 induces apoptosis in human umbilical vein endothelial cells and in glomerular- and foreskin microvascular endothelial cells.

V58/V VEROTOXIN INHIBITS MITOGENESIS AND PROTEIN SYNTHESIS IN HUMAN MESANGIAL CELLS WITHOUT AFFECTING CELL-VIABILITY.

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Endothelial damage in the glomeruli/arterioles of the kidney induced by verotoxin (VT) is believed to play a crucial role in the pathogenesis of HUS. Little information is available regarding the effects of VT on mesangial cells (MC). We investigated the effects of VT on MC in vitro. MC were enriched by collecting hillock-shaped outgrowths derived from adult glomeruli, and subsequently purified by elimination of epithelial cells by immunoseparation with UEA-I-coated dyna-beads. The obtained MC populations were >98% pure as determined by the presence of α-SM cell actin and absence of cytokeratin and CD31. MC bound VT to bands of globotriaosylceramide (Gb3) and a closely related glycolipid, which is similar to a glycolipid involved in the VT-dependent cytokine production in monocytes. VT did not induce the release of cyto/chemokines in MC. In other VT susceptible cells , binding of VT to Gb3 causes cell death by inhibition of protein synthesis. Although protein synthesis was inhibited in MC all cells remained viable. Furthermore, VT markedly inhibited mitogenesis in MC. This inhibition was also found with the B-subunit of VT alone, albeit to a lesser extent, without affecting protein synthesis. Because the inhibition of protein synthesis involves the A-subunit, this suggests that two distinct pathways contribute to the effects of VT in MC.

URINARY LEVELS OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) AND INTERLEUKIN-8 (IL-8) ARE ELEVATED IN HUS PATIENTS

V59/V

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Inflammatory mediators (IF) appear to play a pivotal role in the pathogenesis of HUS. In vitro, verotoxin (VT) cytotoxicity in human glomerular endothelial cells requires the additional pre-exposure to IF. In vivo, predominantly urinary levels of IF are elevated. Monocytes (MO) which upon exposure to VT in vitro release a variety of IF, may be the culprits. The molecular mechanism for the recruitment of MO and neutrophils (PMN), the latter also implicated in endothelial cell damage by release of proteases, is unclear. We studied the presence of two prime candidates (MCP-1 and IL-8) in urine of 15 HUS patients by ELISA. Furthermore, kidney biopsies of HUS patients were examined for MO infiltration (CD14) and MCP-1 expression. Chemokines were below detection limit in urine of 17 controls but were significantly elevated in serial samples of all HUS patients (n=15). In patients with mild renal disease (n=5) the mean of the highest chemokine levels measured during the course of the disease were 1700 (MCP-1) and 122 (IL-8) ng/mmol creatinine. In patients with either moderate or severe renal disease (n=10) the means were 3855 (MCP-1) and 194 (IL-8) ng/mmol creatinine. Immunohistochemical studies revealed MO infiltration as well as MCP-1 expression in glomeruli of HUS patients. These data suggest a local role for MCP-1 and IL-8 in the pathogenesis of HUS, possibly through the recruitment and activation of MO and PMN, respectively.

MECHANISMS OF NEUTROPHIL MEDIATED DAMAGE TO VEROCYTOTOXIN-1 (VT-1) EXPOSED HUMAN GLOMERULAR CAPILLARY ENDOTHELIAL (GCEC) AND UMBILICAL VEIN ENDOTHELIAL (HUVEC) CELLS.

V78/V

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Since leukocytosis and neutrophilia predict for poor prognosis in HUS, a role for neutrophil (N) mediated damage to glomerular cells has been proposed. To determine whether N injure VT-1 treated EC by apoptosis or necrosis, human GCEC and HUVEC were exposed to 0.01-1nM VT-1 and incubated with N for up to 2 hr. Adherence of N to VT-1 treated EC was increased in a dose and time dependent manner. VT-1 was toxic for both EC; after N incubation, additional cell damage and increased EC detachment was seen in VT-1 treated cells. VT-1 treated EC exposed to N had no increase in the degree or frequency of apoptosis. N induced more cell injury and cell detachment in EC exposed to VT-1. The EC death appeared to be due to necrosis. Neutrophils may augment the development of glomerular capillary thrombosis and injury in HUS.

V79/V

ROLE OF CASPASES (CYSTEINE PROTEASES) IN VEROCYTOTOXIN-1 (VT-1) MEDIATED APOPTOSIS

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VT-1 is cytotoxic for human glomerular cells and may induce apoptotic cell death in susceptible cells. To further define the mechanisms of apoptosis with VT, the role of interleukin-1 converting enzyme (ICE) was studied. Vero cells were exposed to 1-100 pM VT-1 for 6-48 hr; cytotoxicity and apoptosis (acridine orange uptake, EM, DNA ladder) were assessed. The role of ICE was evaluated with ZVAD, an aldehyde ICE inhbitor. Cell death and apoptosis (9.6% with 100 pM/24hr) was seen with VT-1. By 48 hr more cytotoxicity but less apoptosis was detected. Vero cells contained ICE protein and ZVAD blocked 73% of apoptosis triggered by VT-1. Apoptosis initiated in Vero cells by VT-1 appears to be partially dependent on ICE. The role of other caspases remains to be determined. Identification of the mechanisms of apoptosis initiated by VT-1 may provide novel strategies to prevent cellular injury in children with HUS.

V82/V

EFFECTS OF VEROCYTOTOXIN-1 (VT1) ON PRIMARY HUMAN RENAL CELL CULTURES.

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This study compares the effects of VT1 on primary human renal and vero cell cultures. Protein synthesis was measured by ^{35}S-methionine uptake, DNA fragmentation by the diphenylamine assay and viability by the MTT assay. Assays were at 24h using 100pg/ml VT1 except for MC which used 100ng/ml. VT1 inhibits protein synthesis in primary cultures of glomerular epithelial (GEC), cortical tubular epithelial (CTEC) and mesangial cells (MC), with a significantly greater effect in GEC and CTEC than in Vero cells. However, unlike Vero cells, apoptosis is not apparent. Using the MTT assay for viability decreases could be seen in GEC and CTEC, but not MC.

Cells	% protein synthesis	% DNA fragmentation		% viability
		basal	VT1	
Vero	14.3±1.9	0.84	29.8	69.94
GEC	1.7±0.3	0	2.8	82.6
CTEC	0.9±0.4	10.8	12.3	70.2
MC	74.8±1.3	10.8	10.5	107.13

VEROTOXIN SPECIFICALLY TARGETS PRIMARY AND SECONDARY OVARIAN TUMOURS AND THEIR VASCULATURE V93/V

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Despite its role in the etiology of HUS, VT1 has been identified as a potential antineoplastic agent[1].We have documented the increased expression of the verotoxin receptor, Gb_3 in 39 ovarian tumors as a function of tumour type and degree of differentiation. Gb_3 is significantly elevated in all ovarian carcinomas but markedly increased in ovarian metastases, particularly in slower tlc migrating species, corresponding to short chain fatty acid Gb_3 isoforms. Toxin overlay of frozen sections from primary and secondary tumors show the toxin specifically binds to tumour cells and cells within the luminal margin of tumour-associated microvasculature. Toxin binding is reduced for highly differentiated tumours except in the case of multiple drug resistance when extensive toxin binding is seen. VT staining of blood vessels adjacent to ovarian metastases to the colon was observed. No staining of blood vessels within the normal ovary or colon was seen. These studies demonstrate that verotoxin targets undifferentiated ovarian tumour cells and may provide the basis for a new antiangiogenic and antineoplastic approach to the therapy of ovarian cancer.

1.-Farkas-Himsley H.,*et al* Proc Natl Acad Sci 92:6996-6999 (1995)

VEROTOXIN AS AN ANTINEOPLASTIC AGENT: TREATMENT OF HUMAN ASTROCYTOMAS XENOGRAFTS IN NUDE MICE V98/V

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Despite its role in the etiology of HUS, VT1 has been identified as a potential antineoplastic agent[1].VT receptor (Gb_3) expression is elevated in certain human neoplasias. We have found that certain astrocytoma cell lines are particularly sensitive to verotoxin in vitro[2]. Morphological evidence of VT1-B subunit induction of apoptosis in cultured astrocytoma cells can be detected 90 min. after addition. Nude mice bearing subcutaneous human astrocytoma xenografts were treated with a single intratumour VT1 injection. 100% Tumour regression was observed without relapse >50days. Samples taken 24 hours after toxin administration indicated massive induction of apoptosis in both the tumour and its vasculature. >50% Decrease in tumour size was observed 5 days post-treatment and animals were tumour free within 10-20 days. These results support the potential of VT1 as a novel approach to cancer therapy.

1.-Farkas-Himsley H.,*et al* Proc Natl Acad Sci 92:6996-6999 (1995)
2-Arab,S.*et al* Neuropath Exp Neurol in press

V105/V A MURINE MODEL FOR HEMOLYTIC UREMIC SYNDROME

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In an attempt to produce an animal model of HUS, 16 Swiss albino mice were injected intraperitoneally with graded doses of SLT-II. The kidneys appeared normal in conventional paraffin sections. On ultrastructural study there was dose dependent glomerular endothelial damage with occasional platelet clusters and fibrin tactoids. These glomerular endothelial lesions, similar to that in human HUS have been produced in an animal model for the first time.

V114/V 8-ISOPROSTANE (8-*EPI*-$PGF_{2\alpha}$) CONCENTRATIONS IN THE URINE OF CHILDREN WITH POST-DIARRHEAL HEMOLYTIC UREMIC SYNDROME (HUS)

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The non-cyclooxygenase derived prostanoid 8-*EPI*-$PGF_{2\alpha}$ results from oxidation of arachidonic acid containing lipids in plasma, membranes and the kidney, and is therefore considered a marker of lipid peroxidation. Since there is evidence of both oxidative injury and renal vasoconstriction in post-diarrheal HUS, we measured urinary 8-*EPI*-$PGF_{2\alpha}$ in 10 children during the acute phase of post-diarrheal HUS and at convalescence. Their samples were compared to those of 8 healthy control subjects and 6 who had acute renal failure (ARF) from causes other than HUS. We used a competitive enzyme immunoassay (EIA) (Cayman Chemical Co.) preceded by ether extraction. Values (mean + SEM) are expressed as pg/mg of urinary creatinine.

Acute HUS	At Recovery	Controls	Non-HUS ARF
2835 ± 1011	12473 ± 837	7030 ± 1033	2099 ± 157

8-*EPI*-$PGF_{2\alpha}$ values during the acute phase of HUS were significantly lower ($p=.03$) than those of normal control subjects, rose to supranormal levels ($p=.007$) at convalescence, and were not significantly different from those with renal failure from other causes. These observations argue against intrarenal oxidative injury, fail to support a role for 8-*EPI*-$PGF_{2\alpha}$ in the pathogenesis of HUS ARF and suggest that acute renal damage suppresses renal 8-*EPI*-$PGF_{2\alpha}$ production.

SHIGA TOXIN ACTION ON HUMAN INTESTINAL MICROVASCULAR ENDOTHELIAL CELLS (HIMEC) V144/V

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Endothelial cell (EC) damage is considered to be the primary mechanism of action of Shiga toxin (Stx) resulting in thrombotic microangiopathy in the intestine and kidney. In order to elucidate Stx-EC interaction in the intestine, we have examined the effects of Stx on primary and transformed HIMECs. Both had typical endothelial cell characteristics and responded to LPS and TNFα by increased surface VCAM-1, ICAM-1 and E-selectin expression, and IL-8 secretion. Stx 1 or 2 resulted in significant inhibition of protein synthesis in HIMECs (ID_{50} approximately 1-10 pg/ml), although preexposure of HIMECs to LPS, IL1 or TNFα did not increase their sensitivity to Stx. HIMECs contained about 3 times as much Stx receptor (Gb3) as HeLa cells (determined by HPLC) and bound more iodinated Stx1 and 2 than either HeLa or Vero cells. In conclusion, HIMECs are extremely sensitive to Shiga toxins and will provide a useful model to examine intestinal epithelial cell-Shiga toxin-endothelial cell interactions.

EFFECTS OF SHIGA-LIKE TOXIN (SLT-1) ON HUMAN MESANGIAL CELLS V146/V

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Human mesangial cells (HMC) are potential targets of injury in HUS and progressive glomerulosclerosis is a well recognized complication of HUS. We determined the effect of SLT-1 on protein-synthesis in HMC using ^{35}S-labeled methionine/cysteine and on cell viability using the neutral red assay. Incubation of HMC for 24hrs with SLT-1 (0.24ng/ml-2.4μg/ml) resulted in a dose-dependent inhibition of protein-synthesis, an effect that was potentiated by preincubation with IL-1α [2ng/ml]. The effect could be seen as early as 1hr after incubation with SLT-1. Cell viability exceeded 95% after 24hrs of incubation with SLT-1. However, similar incubations for 48hrs and 72hrs showed a 68% and 80% decrease in cell-viability, respectively. SLT-1 also resulted in a dose and time dependent stimulatory effect on MCP-1 mRNA abundance, an effect that was potentiated by preincubation of HMC with IL-Iα [2ng/ml]. These data provide evidence, that HMC are susceptible to the effects of SLT-1 in vitro. Immunoinflammatory cytokines potentiate the effect of SLT-1 on these cells. Thus the glomerular pathology in HUS may also result from direct effect of SLT-1 on mesangial cells.

V148/V

VEROTOXIN 1 ACCELERATES PMN MEDIATED HUMAN GLOMERULAR ENDOTHELIAL CELL (GEC) INJURY

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Clinical studies suggest that oxidants play a role in GEC injury in HUS. To determine the role of PMNs and VT-1 in mediating injury, we exposed GEC in vitro to 250 U/ml TNF for 24 hrs followed by 24 hr with 0, 0.1, 1.0 or 10 pM VT-1. Treated GEC were then exposed to 5000/mm^3 activated PMNs; ATP levels (sublethal injury) were determined by luciferin-luciferase. ATP levels (pmol/mg protein) in control GEC were $9.0 \pm .3$, $8.9 \pm .3$ in GEC + TNF, $9.1 \pm .3$, $8.5 \pm .3$, $8.5 \pm .2$ in GEC + TNF with 0.1, 1.0, or 10 pM VT1 (w/o PMNs), respectively. When identically treated GEC were exposed to PMNs, ATP levels were $7.7 \pm .3$ in control cells, $7.1 \pm .7$ in GEC + TNF, and 7.7 ± 1.2, $4.9 \pm .3$, $2.9 \pm .8$ in GEC + TNF with 0.1, 1.0, or 10 pM VT-1, respectively. We conclude that VT-1 greatly accentuates PMNs mediated GEC ATP depletion and sublethal injury.

V151/V

VEROTOXIN (VT) I, II AND LPS ARE TOXIC AND INDUCE APOPTOSIS IN RENAL EPITHELIAL CELLS (LLC-PK_1)

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To elucidate the mechanism of toxity we studied the effect of VT I, II and LPS on function, proliferation and cell death on LLC-PK1 cells in culture. Toxity was evident by fall in transepithelial resistance (TER), reduction in cell proliferation (BrdU-incorporation). Apoptosis was evaluated by nuclear condensation and DNA-laddering and loss of cell polarity by immunofluorescence to α-catenin (zonula adherens). TER decreased significantly already after 6h by VTI>VTII>LPS, comparable to the degree of decrease in BrdU-incorporation. After 24 h, in addition, cell contacts were lost (loss of polarity) and profound DNA-laddering was evident indicating preferentially apoptosis. These results indicate, that verotoxin is not only toxic to endothelial cells but also to epithelial cells. Thus, renal insufficency associated with EHEC may be a consequence of tubular as well as endothelial cell injury.

DYSFUNCTION OF von WILLEBRAND-FACTOR (vWF) IN CHILDREN WITH HEMOLYTIC UREMIC SYNDROME

V152/V

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vWF is a marker for endothelial cell injury. In children with HUS the vWF was evaluated as antigen (AG) and a new functional assay (CBS). Patients with EHEC positive history (18) were compared to EHEC negative (4), renal insufficiency (6) and patients with gastroenteritis (3). vWFAG was 2.8, CBS was 2.07), the ratio AG/CBS 0.75). In contrast, patients with gastroenteritis have a vWFAG of 1.17, CBS of 1.84 and ratio of 1.58. Children with renal failure not caused by HUS have a vWFAG of 1.45, CBS of 1.44 and ratio of 1.06. Abnormalities in the multimeric structure were not found. AG/CBS of 0.79±0.16 from typical was not different from atypical HUS (0.60±0.08). Thus, patients with HUS clearly are distinct from normals, from children with diarrhea and renal failure in regard to their ratio of functional to immunological vWF reflecting the degree of endothelial cell injury.

INDUCTION OF SHIGA-LIKE TOXIN SENSITIVITY IN ENDOTHELIAL CELLS INVOLVES PROTEIN KINASE C, BUT NOT NF-κB.

V173/V

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In enterohemorrhagic *E. coli*-associated disease of humans, TNF-α, IL-1β, and bacterial LPS sensitize endothelial cells (EC) to Shiga-like toxin (Stx1). Yet, little is known how these agents induce Gb3 expression on the EC surface. Phorbol myristate acetate (PMA) is a protein kinase C (PKC) activator that also sensitizes ECs to Stx1. In the present study, it was demonstrated that inhibitors of PKC enzymes interfered with PMA- and LPS-induction of Stx1 sensitivity, but had no effect on TNF- and IL1-induction of Stx1 sensitivity. These data were obtained using site-specific inhibitors of class I, II and III PKC enzymes. We also examined the role of transcriptional activation factor NF-kappa B in the induction process of Stx1 sensitivity. While TNF treatment resulted in the activation of NF-κB, the presence of a NF-κB inhibitor during TNF treatment did not interfere with the induction of Stx1 sensitivity. These results indicate that PKC is utilized by LPS, but not by TNF and IL-1 during induction of Gb3 in human umbilical endothelial cells, and that NF-κB is not required by any of these factors during the induction process.

V174/V EFFECT OF CERAMIDE METABOLISM ON SHIGA TOXIN RECEPTOR (GB3) AND TOXIN SENSITIVITY IN HUMAN ENDOTHELIAL CELLS.

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The action of Shiga toxins (Stx1, Stx2) in vascular disease caused by *E. coli* O157:H7 is believed to occur particularly at the endothelial cell (EC) level. It is generally accepted that targeting of Stx is determined by the quantity and quality of the receptor, Gb3, on individual cell types. In the present study, three different agents that increased ceramide content in ECs all sensitized ECs to Stx1. The order of activity was sphingomyelinase>C8 ceramide>N-oleoylethanolamine. The latter compound is an inhibitor of ceramidase that combined with TNF-alpha to yield an additive increase in Gb3. Sphingomyelinase did not induce ceramide:glucosyltransferase mRNA, indicating that the regulation of Gb3 was due to substrate (i.e. ceramide) limitation. It was observed that all forms of Stx sensitivity were prevented when ECs were preincubated for 24 hours with glycosyltransferase enzyme inhibitors (PDMP, PPMP, PPPP) of the Gb3 pathway. These data suggest that in some cases, ceramide is rate-limiting for Gb3 synthesis in ECs.

V175/V TRANSCRIPTIONAL REGULATION OF GB3 PATHWAY GLYCOSYLTRANSFERASE ENZYMES IN HUMAN ENDOTHELIAL CELLS.

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The action of Shiga toxins (Stx) on endothelial cells (EC) in the development of HUS may require induction by LPS and cytokines of the enzymes responsible for Gb3 synthesis. To investigate this, a 1.34 kb cDNA encoding human ceramide:glucosyltransferase (CGT), was cloned into pBluescript-SK+. A 442 bp NsiI-SnaBI fragment from this clone containing the 5' region of the gene was used to probe total RNA prepared from human endothelial and epithelial cell lines treated with various inducers of Stx-sensitivity. Northern blot analysis showed a CGT mRNA transcript of ~4 kb in all cells. Although phorbol myristate acetate (PMA) induced Stx sensitivity in all endothelial and epithelial cells tested, a concomitant induction of CGT mRNA was observed in only some cell cultures. The inductions of Stx sensitivity by PMA were both time- and dose-dependent. These results suggest that induction of Stx sensitivity in some, but not all ECs, may be transcriptionally regulated at CGT, the first glycosyltransferase enzyme involved in Gb3 synthesis.

VEROTOXIN CAUSES CYTOTOXICITY IN HUMAN CEREBRAL ENDOTHELIAL CELLS **V190/V**

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The role of cerebral endothelial cells in the pathophysiology of the encephalopathy of hemolytic uremic syndrome is unknown. We hypothesized that Verotoxin-1 would cause cytotoxicity in human cerebral endothelial cells (HCEC) *in vitro* and that this cytotoxicity would be enhanced by TNFα and IL-1β. Human cerebral endothelial cells were coincubated for 24h with Verotoxin-1 at various concentrations with and without TNFα or IL-1β. Cytotoxicity was quantified using vital dye (CFDA-AM) exclusion and propidium iodine (killed cells) staining with a cytofluor analyser. Verotoxin-1 caused increasing cytotoxicity in HCEC at concentrations from 0.1 to 100 nM and the toxicity was enhanced by TNFα (100u/ml) and IL-1β (100u/ml). In conclusion, verotoxin-1 causes cytotoxicity in human cerebral endothelial cells and the cytotoxicity is enhanced by coincubation with TNFα and IL-1β.

ENDOTHELIAL CELL ACTIVATION BY VEROTOXINS: NOVEL EFFECTS ON VASOMEDIATOR EXPRESSION **V201/V**

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A paucity of data is available on the endothelial genes implicated in the pathobiology of verotoxin (VT)-associated hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). We defined the effects of VTs on the expression of potent endothelial-derived vasomediators, endothelin-1 (ET-1) and nitric oxide (NO), using bovine aortic endothelial cells. VT1 and VT2, but not receptor-binding VT1 B-subunit induced an increase in steady state preproET-1 mRNA transcript levels in a dose- (0.1 to 10 nM) and time-dependent fashion (peak at 12 - 24 h), at concentrations that had trivial effects on [^{3}H]leucine incorporation. In contrast, endothelin converting enzyme-1 and endothelial constitutive NO synthase (ecNOS) mRNA transcript levels remained unchanged as did levels of ecNOS protein expression and calcium-dependent NOS enzymatic activity. The mechanism underlying VT-induced increases in preproET-1 mRNA levels was investigated using nuclear transcription assays. Results indicated that VTs acts by stabilization of labile preproET-1 mRNA transcripts. VTs can directly activate endothelial cells in the absence of exogenous cytokines. Perturbed expression of endothelial-derived vasomediators may play a pathophysiologic role in the microvascular dysfunction that is the hallmark of HUS and HC.

V206/V

RECRUITMENT OF RENAL TUBULAR EPITHELIAL CELLS EXPRESSING VEROTOXIN-1 (VT-1) RECEPTORS IN HIV-1 TRANSGENIC (HIV-Tg) MICE.

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Children infected with HIV-1 are at risk of developing HUS-TTP. The pathogenesis of HIV-associated HUS is obscure. Recent studies have shown that interleukin 1β and TNF α upregulate the expression of verotoxin receptors (Gb_3) in human endothelial cells. These cytokines are present at high systemic levels in HIV-1 infected children. To determine whether the HIV-1 "cytokine milieu" modulate the expression of renal Gb_3 receptors *in vivo*, we utilized transgenic mice expressing a deletion mutant of HIV-1 (pNL4-3). These mice develop some clinical features similar to those present in HIV-1 infected children, including renal disease. Kidney sections were stained with FITC-labeled VT-1 and anti-Gb_3 antibody using immunohistochemistry techniques. Glomerular endothelial cells from control and HIV-Tg mice were unlabeled. HIV-Tg disease kidneys however, showed a significant recruitment of renal tubular epithelial cells (RTEc) expressing VT-1 receptors in renal cortex and medulla. VT-1 staining in control kidneys was limited to tubular collecting ducts. Binding of VT-1 was significantly reduced by pretreating the kidney sections overnight with α-galactosidase. Moreover, VT-1 TLC overlay studies of lipid extracts from control and HIV-Tg kidneys, confirmed the presence of elevated Gb_3 levels in HIV-Tg kidneys. Recruitment of VT-1 receptors in RTEc was associated with high levels of inflammatory cytokines. HIV-1 infected children with renal disease may have an increased number of VT-1 receptors in renal tubules and may be more sensitive to VT cytotoxic effects. Thus, when exposed to Shiga-like toxins, they may be at risk of developing an atypical HUS with severe tubular injury and rapid progression to end stage renal disease.

V207/V

INCREASED RELEASE OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN CHILDREN WITH CLASSIC HEMOLYTIC UREMIC SYNDROME (HUS).

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The ability of Shiga-like toxins to injure renal microvascular endothelial cells is a primary requisite for the development of HUS. We hypothesized that growth factors released during endothelial injury may play a relevant role in the pathogenesis of classic HUS. In a preliminary screening of vascular growth factors in the urine of ten children affected with classic HUS, we found significant levels of bFGF-like activity. Further purification of the most active fractions by heparin Sepharose column chromatography revealed an 18 kDa band consistent with the presence of bFGF. By radioimmunoassay, we found elevated levels bFGF in HUS affected children (156 ± 24 pg/ml) when compared to control children (12 ± 4 pg/ml. $P < 0.005$). Immunohistochemistry studies using specific bFGF antibodies revealed an increased expression of bFGF in renal cortex, medulla and extracellular matrix surrounding renal tubules, only in HUS affected kidneys. Renal sections obtained during the acute stage of HUS demonstrated an increased number of bFGF low affinity binding sites in renal glomeruli and medulla. Since many Argentinean children develop focal segmental glomerulosclerosis after HUS, we determined the *in vitro* effects of bFGF on renal vascular contractility and mesangial cell growth. FGF (1-10 ng/ml) stimulated contraction of cultured microvascular smooth muscle cells and proliferation of mesangial cells. Thus, the renal accumulation of bFGF may play an important pathogenic role during the acute stages of HUS and may be one of the mechanisms leading to hyperfiltration and focal segmental glomerulosclerosis in Argentinean children.

DETECTION OF O157 VTEC IN ENVIRONMENTAL SAMPLES FROM A DAIRY BY IMMUNOMAGNETIC SEPARATION (IMS)

V3/VI

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Over 100 people were affected by an outbreak of *E.coli* O157 (O157 VTEC) infection associated with consumption of pasteurised milk from a dairy in the West Lothian area of Scotland; Sixty-nine had faecal isolates of VT2-producing *E.coli* O157:H7 phage-type 2. This is the largest reported milkborne outbreak of *E.coli* O157 infection world-wide, and was the first to involve a heat-treated milk supply. Numerous milk and environmental samples from the dairy were analysed by means of direct culture, and the novel technique of immunomagnetic separation (IMS). Direct culture failed to yield the organism. However O157 VTEC was isolated by IMS from a discarded bottling machine rubber, a pipe leading from the pasteurisation apparatus to the bottling machine, and a bulk milk sample from the feeder dairy farms. The environmental isolates were indistinguishable by phage-typing and PFGE analysis from each other, from the clinical isolates, and from bovine faecal isolates from one of the feeder dairy farms.

DETECTION OF O157 VTEC IN CLINICAL SAMPLES BY IMMUNOMAGNETIC SEPARATION (IMS)

V4/VI

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Verocytotoxigenic *E.coli* O157 (O157 VTEC) infection is routinely diagnosed by culture on sorbitol MacConkey (SMAC) agar. Although present in large numbers during the acute diarrhoeal phase, there is a rapid decline as symptoms resolve. This may prevent isolation of O157 VTEC in late-presenting disease, or where the initial presentation is a complication such as haemolytic-uraemic syndrome, which develops when the diarrhoeal phase is resolving. Immunomagnetic separation (IMS) increases culture sensitivity by ten to one-hundredfold, and is invaluable in the detection of O157 VTEC in contaminated foodstuffs. We have used IMS to isolate O157 VTEC from 21 faecal samples from 20 patients which failed to yield the organism by direct culture. We recommend this technique for the diagnosis of O157 VTEC infection in late-presenting disease, if there is a high index of clinical suspicion despite negative direct culture results, or in circumstances where it is highly desirable to obtain an isolate of the organism for any reason.

V5/VI GENOMIC TYPING OF *E. COLI* O157:H7 BY SEMI-AUTOMATED FLUORESCENT AFLP ANALYSIS

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E. coli O157:H7 isolates were analyzed using a new DNA typing method, Amplified Restriction Fragment Polymorphism (AFLP). This technique involves digestion of the genomic DNA with restriction endonucleases followed by ligation of oligonucleotide adapters. Subsets from the total pool of restriction fragments are then amplified using one unlabeled and one fluorescently labeled primer consisting of the adapter sequence plus 0-2 selective nucleotides. Amplified fragments were detected and sized automatically on an automated DNA sequencer. Fifty isolates of *E. coli* O157:H7 from food, cattle and humans were analyzed by AFLP using three sets of selective primers. Compared with pulsed-field gel electrophoresis, AFLP provided greater genetic resolution and may prove to be a useful technique for relatively robust subtyping of bacterial pathogens in epidemiologic studies.

V20/VI SHIGA TOXIN PRODUCING *E. COLI* (STEC) IN HEALTHY RUMINANTS AND THEIR VIRULENCE FACTORS

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Feces samples from 336 calves, 111 cows and 134 sheep of different farms were investigated for harbouring Shiga Toxin genes (stx) directly by PCR. 25 % of cows´ samples, 18 % of calves´ samples and 20 % of samples from sheep gave positive results. The positive feces samples were cultured on Biosynth™ medium and from these 108 strains were identified as STEC. These were serotyped and tested for other virulence factors (eaeA, EHEC-Hly and pCVD419-gene) by PCR. *E. coli* O157 were found 3 times in bovine samples, from which one strain of a calf carrying stx_2, eaeA, EHEC-Hly, pCVD419 and not fermenting sorbitol. The other two strains were found to carry stx_2 alone or stx_{1+2} and EHEC-Hly as well as pCVD419. 6 strains were known as O26, only harbouring stx_{1+2} or stx_2. From 4 strains of serotype O101, only one was found with the genes for EHEC-Hly and pCVD419. A single strain of serotype O111 was observed carrying stx_2, EHEC-Hly and pCVD419. The remaining 94 strains were belonging to different serotypes, 10 of them harbouring eaeA, and 39 strains carrying EHEC-Hly as well as pCVD419.

EVALUATION OF THE LMD ELISA FOR DETECTION OF SHIGA-LIKE TOXIN OF *ESCHERICHIA COLI*

V38/VI

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We evaluated a new ELISA test (LMD, Sunnyvale, CA) which detects SLT I and II directly from stools or from MacConkey broth cultures in just 45 minutes. The assay was compared with Premier EHEC (Meridian, Cincinnati, OH) which detects SLT I and II in 2.5 hours. Samples included stools from 306 different patients collected from June-December, 1996 (6 of these were positive) and 34 SLT positive stools stored at -70C. The sensitivity of this LMD assay by direct stool test was 80% (32/40 positives detected) compared with 73% (27/40) with Premier. Specificities were 98% (295/300) for LMD and 100% (300/300) for Premier. Both LMD and Premier were 100% specific and 100% sensitive by the broth culture method. There were 7 SLT producing *E. coli* which were non-O157; both devices detected 5 of these by direct stool test. We conclude that diagnostic efforts should not be limited to detection of O157:H7, since SLT-positive, non-O157 serotypes do exist in this population and can cause hemorrhagic colitis and hemolytic uremic syndrome. The LMD ELISA is rapid and easy to use.

DETECTION OF VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* IN HUNGARY

V39/VI

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The objective of this study was to find tests suitable for proving the etiological role of verocytotoxin-producing *E.* coli (VTEC). Specimens were screened using sorbitol MacConkey (SMAC), Cefixim and tellurit (CT) SMAC and Entero-haemolysin (Ehly) agar, and tested for serotypes, phage types and VT production (Verotox-F, VTEC Screen, Premier EHEC, DNA probes, PCR). Altogether 4532 faeces were tested for the presence of *E. coli* O157 and 545 *E. coli* strains, 526 mixed cultures, 49 faeces were examined for VT. Thirty seven *E. coli* O157 were isolated. Canadian phage types 8, 14, and 31 were observed among our VT-positive O157 isolates. Eighteen non-O157:H7 VTEC were detected: O26:H11 (4), O18ab:H- (2), O39:H48 (1), O76:HNT (2), O157:H7 (2), O157:H33 (1), O157:HNT (3), O157:H- (3). Eleven strains produced VT1, two VT2 and two VT1 plus VT2. VT positivity were verified only by DNA probes in 3 strains. Free VT was detected in 12 faeces; 7 mixed cultures and 4 isolates from these specimens were VT-positive also. CT-SMAC proved to be selective both for O157 and for non-O157 VTEC. VT production by mixed culture is detectable with latex agglutination by our method. Successful VT diagnosis can be expected from different kinds of simultaneously-performed methods.

V45/VI USE OF CHROMAGAR O157 AND RAINBOW AGAR O157 TO ISOLATE SHIGA-LIKE TOXIN-PRODUCING *ESCHERICHIA COLI* O157:H7 FROM SAMPLES OF MEAT AND FAECAL SPECIMENS

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Two new commercial media CHROMagar O157 and Rainbow agar O157, designed specifically to identify and characterize strains of *Escherichia coli* O157:H7, have been used to isolate *E. coli* O157:H7 from samples of meat and faecal specimens deliberately spiked with various concentrations of a pure culture of *E. coli* O157:H7. Sorbitol MacConkey (SMAC) Agar was used for comparison. The pure culture was also spread on these media so that effects on the recovery of the *E. coli* O157:H7 of the normal flora of the faeces or meat could be ascertained. Both media were far superior for the isolation of *E. coli* O157:H7 than the SMAC agar, particularly at low concentrations of *E. coli* O157:H7. The Rainbow agar was slightly better than the CHROMagar. On all three media significant interference with the growth of the *E. coli* O157:H7 was noted compared to the pure cultures of the *E. coli* O157:H7. Both media are useful additions to the laboratory for the isolation of *E. coli* O157:H7. It should be noted that they are not designed to characterise other serotypes of enterohaemorrhagic *E. coli.*

V51/VI VIRULENCE PROPERTIES OF *ESCHERICHIA COLI* FROM CHILDHOOD DIARRHEA IN ITALY

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E.coli strains isolated from 426 children with diarrhea and 103 asymptomatic controls during a study of childhood diarrhea in Italy were examined for phenotypic and genetic characters associated with enterovirulence. Gene probes and PCR amplification were used to detect *eaeA*, EAF, Stx1 and Stx2. LT and ST enterotoxins, and enteroaggregative plasmid (EAgg) gene sequences. Three enterotoxin and 4 Stx-producing strains, one belonging to the enterohemorrhagic serogroup O26, were found among cases. *eaeA*-positive strains were found in 25 cases (5.9%) and 3 controls (2.9%). Nineteen were positive at the HEP-2 cell Fluorescent Actin Staining test, 6 belonged to EPEC serogroups and one was EAF+. EAgg-positive strains were detected in 15 cases (3.5%) and 3 controls (2.9%). Twelve strains showed aggregative adhesion to HEp-2 cells and four belonged to EPEC serogroups (O86, 0111). In conclusion, enterovirulent *E.coli* do not represent a major cause of childhood diarrhea in Italy.

MOLECULAR CHARACTERIZATION OF SHIGA-TOXIN PRODUCING *ESCHERICHIA COLI* O111 FROM DIFFERENT COUNTRIES

V54/VI

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A collection of 31 Shiga-toxin (Stx)-producing *E.coli* (STEC) of serogroup O111 from epidemiologically unrelated cases of diarrhea or HUS in Italy, Germany, and Austria, and from outbreaks occurred in France and Australia were studied to assess their possible clonal relationships. Strains were compared using Stx genotypes, adherence properties, DNA restriction fragment length polymorphisms (RFLP) identified with rRNA and phage λ probes, and repetitive element sequence based PCR. While isolates from Italy, Germany, Austria and Australia showed a remarkable degree of similarity in all the assays, strains from the French outbreak had a different toxin genotype (*stx2* vs. *stx1* or *stx1/2*) and showed aggregative adhesion instead of the typical attaching and effacing mechanism. RFLP and PCR analyses confirmed that STEC O111 have a clonal population structure, with the exception of the French strains which clearly belonged to a distinct bacterial clone.

MEDIA AND TEST KITS FOR THE DETECTION AND ISOLATION OF *ESCHERICHIA COLI* O157 FROM MINCED BEEF

V60/VI

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Selective enrichment and plating media used for the isolation of Verocytotoxin (VT)-producing *E. coli* (VTEC) of serogroup O157 were evaluated by examining pure bacterial cultures. Also, a variety of commercial test kits for the detection of *E. coli* O157 strains inoculated into minced beef was compared (Ampcor *E. coli* O157:H7 Kit, 3M Petrifilm Test Kit-HEC, Dynabeads anti-*E. coli* O157, EHEC-TEK, Tecra *E. coli* O157 visual immunoassay). And finally, the commercial Verotox F test for the determination of VT type of VTEC isolates was compared with a PCR for VT genes. A sensitive and cost-effective procedure for the isolation of O157 VTEC from minced beef in food industry and epidemiological studies involving large numbers of samples is the following: enrichment in modified *E. coli* broth with novobiocin (mEC+n) at 37°C for 6-8 h (100 rpm), followed by immunomagnetic separation using Dynabeads anti-*E. coli* O157 and spread plating of the concentrated target cells onto.sorbitol MacConkey agar with cefixime and tellurite (CT-SMAC). The Verotox F test can be used to determine whether the isolates produce VT1 and/or VT2.

V85/VI APPLICATION OF AFLP FOR THE ANALYSIS OF *E.COLI* 0157 POPULATIONS

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The development of AFLP as a bacterial typing method has allowed us to analyse a range of *E.coli* 0157 isolates using this technique. It involves restriction enzyme digestion with two enzymes, ligation of adaptors and selective PCR of a sub-population of fragments. The amplified fragments were visualised by fluorescent labeling of one of the PCR primers and separated using an ABI automated DNA sequencer. Accurate sizing of the fragments was achieved using internal lane markers. A collection of 26 *E.coli* 0157 were analysed using different restriction enzymes. Outbreak isolates were found to give identical patterns. Differences in banding patterns were observed for sporadic isolates although all isolates had a number of bands in common. This technique may provide a useful method for the identification and characterisation of regions of variation within the genome of *E.coli* 0157.

V89/VI INTIMIN A,B,G,D AND E: FIVE INTIMIN DERIVATIVES EXPRESSED BY ATTACHING AND EFFACING FORMING MICROBIAL PATHOGENS

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Enterohemorrhagic Escherichia coli (EHEC) are divided into two divergent clonal groups. EHEC-1 includes the O157:H7 clone while EHEC-2 composed of shiga-like toxin producing O26:H11 and O111:H8 strains. Likewise, the enteropathogenic E. coli (EPEC) strains also fall into two groups of related clones. Here we describe polyclonal antisera made against the cell binding domain of intimin from EPEC, serotypes O127:H6 and O114:H2. Western blot and immunological analysis revealed that while some strains were poorly recognised by either antiserum, the anti-Int-H6 reacted strongly with strains of EPEC-1, and the anti-Int-H2 reacted strongly with strains of EPEC-2 and EHEC-2. The two intimin derivatives, designated intimin α and intimin β respectively, were also differentially detected using PCR.

THE MICROBIOLOGICAL DIAGNOSIS OF *ESCHERICHIA COLI 0157* INFECTION IN MAN | V103/VI

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We investigated the microbiological diagnosis of *E. coli 0157* infection in a cohort of 48 elderly patients potentially exposed to a point-source. The patients were investigated by culture of faeces onto Sorbitol-MacConkey agar (SMAC) and by immuno-magnetic separation (IMS) technique as well as by immunoblot analysis of patient serum against the lipopolysaccharide (LPS) of *E. coli 0157*. *E. coli 0157* was isolated from faeces of only 3 of 48 patients by the SMAC method compared to 5 patients by the IMS technique. 16 of the 48 patients had IgM antibodies against the LPS of *E. coli 0157* by the immunoblot technique. Sequential samples from patients with evidence of *E. coli 0157* infection were analysed. The significance of these results will be discussed further.

A TRIPLEX PCR METHOD FOR SIMULTANEOUS DETECTION OF SHIGA-LIKE TOXIN GENES AND *ESCHERICHIA COLI* O157:H7 SPECIFIC SEQUENCE IN GROUND BEEF | V112/VI

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Our research focused on developing a PCR procedure that can detect low contamination levels of *E. coli* O157:H7 and other Shiga-like toxin producing *E. coli* (STEC) in beef. Other goals were to compare the PCR procedure to bacteriological culturing and observe whether the fat content of ground beef influences PCR test results. Three target sequences were employed in a multiplex PCR to identify Shiga-like toxin genes 1 and 2 and a portion of the *uidA* gene specific to *E. coli* O157:H7. The sensitivities of PCR and culturing were 90% and 53%, respectively, as determined by analyzing ground beef samples seeded with inoculum 0.14 - 14 CFU/g. Fat content of ground beef did not influence the PCR result statistically. The time required to complete the entire PCR procedure was 7 hours after overnight sample enrichment compared to 52 hours for *E. coli* O157:H7 detection by culturing. The developed PCR procedure could become an alternative procedure for *E. coli* O157:H7 and other STEC detection in ground beef as it was faster and more sensitive than culturing.

V118/VI

SENSITIVITY AND SPECIFICITY OF MERIDIAN IMMUNOASSAY TESTS FOR *E.COLI*

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Stools from diarrhoea patients, 6 mos to 15 yrs, were studied at 8 centres across Canada. Discrepant results were investigated in a reference laboratory. The gold standard was isolation of E. Coli O157:H7. Acute and convalescent sera were tested for O157 antibody. Stools +ve in the EHEC test and -ve for O157:H7 were examined for non-O157 EHEC. *Premier 0157 E.coli* EIA (**EO)** sensitivity in the participating laboratories was 57 / 66 (86%), specificity was 797 / 810 (98%). *EHEC* EIA test for Shiga-like toxin (**EC)** sensitivity was 50 / 56 (89%) specificity was 747 / 751 (99%). 9 of 13 apparently false +ve **EO** tests were -ve when repeated in the reference laboratory, probably resulting from inadequate washing of the wells, and 2 / 4 false positive **EC** tests were also **EO** +ve. Excluding these, the positive predictive values were **EO** (95%) and **EC** (98%). *EHEC* EIA detected 10 non O157 EHEC which would otherwise have been missed. Both these tests are highly sensitive and specific. **EO** is a useful test for accelerated diagnosis of 0157 EHEC. The importance of non-0157 EHEC is becoming apparent, and **EC** is a practical way for routine laboratories to find these organisms. Both tests performed together achieve a very high sensitivity and specificity.

V126/VI

SEQUENTIAL GENOTYPIC AND PHENOTYPIC CHANGES IN THE EMERGENCE OF *ESCHERICHIA COLI* O157:H7

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Escherichia coli O157:H7 is a newly emerged foodborne pathogen that produces potent Shiga-like cytotoxins (STX). It is distinct from *E. coli* by its inability to ferment sorbitol (SOR) and to express β-glucuronidase (GUD) activity. To elucidate the evolutionary emergence of O157:H7, we used an allele-specific probe to examine for genotypic variations in the *uid*A gene that encodes for GUD and used multilocus enzyme electrophoresis to establish clonality among strains. We also looked for phenotypic variations in SOR and GUD expression as well as STX production. Analysis of O157:H7 (SOR [−], GUD [−], STX [+]), non-motile O157:H$^-$ (SOR [+], GUD [+], STX-II) variants implicated in HUS in Germany and enteropathogenic *E. coli* O55:H7 (SOR [+], GUD [+], STX [−]) associated with infantile diarrhea, showed that a G → T base substitution at +92 in the *uid*A was conserved in O157:H7 and its STX-producing non-motile variants, including the O157:H$^-$ strains from Germany. This base change was not found in O55:H7 or other serotypes. A T → A base change in the −10 promotor region of *uid*A was present in O157:H7, O157:H$^-$ and also in O55:H7, but not in other distantly related *E. coli*. The electrophoretic profile of O157:H7 and O55:H7 are similar and distinct from other *E. coli*, while that of O157:H$^-$ resemble O157:H7 with only minor differences. These results support an evolutionary model that O157:H7 evolved sequentially from an O55:H7 ancestor, first by acquiring the STX-II gene, then the gene encoding for the O157 antigen. This toxigenic intermediate then gave rise to two O157 clones; one which lost the ability to ferment SOR and to express GUD (O157:H7) and the other which lost motility and the expression of the H7 antigen (O157:H$^-$).

INCIDENCE OF HUS AND ROLE OF O157 AND NON-O157 VTEC INFECTION IN HUS IN BELGIUM

V130/VI

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To evaluate the incidence of HUS in Belgium and to determine the role of O157 and non-O157 VTEC, 22 centers registered all cases of HUS and when possible collected faecal samples for culture & PCR and serum for LPS antibodies (serotypes O157, O26, O91, O103 & O111). Forty-six cases of HUS (including 5 incomplete cases) were recorded in 36 children (32 post-diarrheic) and 10 adults (5 post-diarrheic). Stools or serum were available from 38 cases. Evidence of VTEC infection was found in 22 children and 1 adult: O157 in 16 cases, O157+O26, O26, O111, O121, O172, O not typable in 1 case each; in one case no isolate was recovered in spite of a positive PCR for VT2. The yearly incidence of complete HUS was at least 4.2 cases/100 000 children < 5 year and 0.4 cases/100 000 inhabitants, comparable to other data from Europe and North America. More than one fourth of the cases were due to non-O157 VTEC, showing that other serotypes also play a role in HUS in Belgium.

AUTOMATIC IDENTIFICATION OF VEROTOXIN-PRODUCING *ESCHERICHIA COLI*. STRAINS

V133/VI

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Verotoxin-producing *E. coli* strains (ECEH) appeared in France in 1991. Isolated ECEH strains belonged to serotypes O157:H7, O116:H21, O103:H2, O111, or O26. Although phenotypic and genotypic schemes were developped for subtyping O157:H7 strains, complete serotyping (O:H) is often unavailable in most laboratories. We attempted to build a ribotyping system that would correlate with serotyping. A collection of 105 (VT1 and/or VT2) ECEH strains was studied by ribotyping with *Mlu*I enzyme and by 99 carbon source utilization tests using BioMerieux Biotype-100 strips. The obtained patterns were analysed using software package Taxotron® (Taxolab, Institut Pasteur, Paris). Serotypes O157:H7, O116:H21, O103, and O22 had characteristic patterns. Our results allowed to establish two databases for ribotyping and biotyping for automatic identification of ECEH strains.

V137/VI PHENO-GENOTYPING OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* STRAINS OF HUMAN AND FOOD ORIGIN IN ARGENTINA.

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Phenotype and genotype of 52 *E. coli* strains isolated from Hemolytic Uremic Syndrome (n=29), hemorrhagic colitis (n=16), nonbloody diarrhea (n=5), intestinal occlusion (n=1) and asymptomatic (n=1) patients were studied. Five *E. coli* isolates from food were also included. All strains were tested by colony blot hybridization with DNA probes for EHEC-adherence factor, VT1 and VT2 and attaching-effacing (*eae*) genes. VT1 and VT2 genes were also detected by PCR. Toxin activity was evidenced by neutralization test using VT1 and VT2 monoclonal antibodies on Vero cells. Serotypes detected were: O157:H7 (53), O157:H- (2), 025:H2 (1), O127:H21 (1). 73.6% of *E. coli* O157:H7 strains belonged to biotype C. Among the strains of this serotype, one isolate of food origin fermented Sorbitol and showed ß-glucuronidase activity. 84.9% of *E.coli* O157:H7 strains were susceptible to all antimicrobial agents tested. 96.4% of *E. coli* O157 strains harbored EHEC, *eae* and VT (98% VT2) genes. Non-O157 *E. coli* strains were positive for EHEC and VT2 genes. The VTs results were confirmed by PCR and Vero cells assays. VT2-producing *E. coli* strains, mainly of serogroup O157, are prevalent in Argentina.

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V139/VI ENHANCED DISCRIMINATION OF *ESCHERICHIA COLI* FLAGELLA USING MONOCLONAL ANTIBODIES AND DNA SEQUENCE DATA

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According to published data, within the group of 54 identifiable *E. coli* flagellar antigens, the serotypes of H2 and H7, regardless of O type, are among the five most frequent H-types isolated from human EPEC, EHEC and ETEC patients. However, the conventional tube agglutination test using polyclonal antibody to classify H2 and H7 often react with the nonhomologous antigen on the standard strains. Specifically, H2 cross-reacts with H7, 12, 23, 28, 38, 44, 51 and 56. H7 cross-reacts with H12, 27, 28, 41, 44, 51 and 56. Surprisingly, the true "H" was discriminated often by conventional wisdom based upon the level of agglutination activities. Identification of the epitopes for each "H" by monoclonal antibody or detection of specific sequences for each "H" and circumvent induction of motility to identify flagella type will allow precise typing of *E. coli*. Because a limited number of flagella serotypes are associated with pathogenic O groups, accurate identification is important in diagnostics and epidemiological studies. Therefore, we have developed monoclonal antibodies specific to *E. coli* H2 and H7 flagella and, more importantly, have identified the minimal sequences which define the sero-specificity for H2 and H7.

ENTEROHEMORRHAGIC *ESCHERICHIA COLI* SHARE A COMMON SURFACE EPITOPE

V140/VI

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Enterohemorrhagic *Escherichia coli* (EHEC) have been defined based on the presence of genes for Shiga-like toxins, intimin and enterohemolysin, along with association with specific disease syndromes. However, these genes may be present in non-EHEC *E. coli*, complicating interpretation of diagnostic and screening results. By screening a panel of monoclonal antibodies generated against *E. coli* O157:H7, we have identified an antibody which reacts with 88% of EHEC isolates tested including 12/12 O157:H7/NM, 5/5 O111:H8/NM and 5/5 O26:H2/H11/NM. Low level expression of the determinant detected by this antibody was detected in some enteropathogenic and enteroaggregative isolates, notably O55:H7, the putative ancestor of O157:H7. The antibody provides an additional marker for EHEC which may be simply applied in a variety of immunologic assay formats. The nature and genetic basis for the epitope detected by this antibody are under investigation.

RAPID IDENTIFICATION OF *ESCHERICHIA COLI* O157:H7 IN BROTH USING A PANEL OF THREE MONOCLONAL ANTIBODIES

V141/VI

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Monoclonal antibodies (MAbs) generated against lipopolysaccharide (LPS) O-chain and core and against flagella of *E. coli* O157:H7 were used to accurately serotype bacterial monocultures. Isolates were grown in trypticase soy broth (TSB), heat killed, and directly coated onto replicate wells of microtiter plates. Enzyme-linked immunosorbent assay (ELISA) reactivity following incubation with the anti-O157, anti-LPS core, and anti-H7 MAbs in separate wells permitted independent determination of the presence or absence of the O157, a possible enterohemorrhagic *E. coli* marker, and the H7 antigens on an isolate. A positive reaction in all 3 wells confirmed the isolate as *E. coli* O157:H7. The 3 MAb ELISAs had 100% specificity, detected an inoculum of 1 bacterium after 9 h growth at 37°C in TSB, and identified *E. coli* O157:H7 in a 100-fold excess of non-target bacteria and in artificially contaminated meat. Use of the anti-H7 MAb in an anti-motility assay allowed further verification of H7 expression by *E. coli* isolates.

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DEVELOPMENT OF A COMPETITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY (cELISA) FOR DETECTION OF SERUM ANTIBODIES TO O157 ANTIGEN OF *ESCHERICHIA COLI*

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The O157 antigen of *Escherichia coli* shares structural elements with lipopolysaccharide (LPS) antigens of other bacterial species, notably *Brucella abortus* and *Yersinia enterocolitica* O9. These similarities confound interpretation of assays for anti-O157 antibodies. To address this problem a monoclonal antibody specific for O157, designated MARC 13B3, was derived which did not cross-react with *Brucella* or *Yersinia* spp. A cELISA was designed using highly purified *E. coli* O157:H7 LPS as antigen and MARC 13B3 as the competing antibody. The cELISA had greater sensitivity and specificity than the indirect ELISA (iELISA) detecting anti-O157 antibodies in sera from cattle experimentally inoculated with O157:H7. Sera from naive heifers had no detectable anti-O157 titers by cELISA before or after *Brucella abortus* Strain 19 vaccination while 30% of pre-vaccination and 75% of post-vaccination sera were positive by iELISA. The cELISA is a sensitive and specific method for the detection of serum antibodies caused by exposure to *E. coli* O157.

V153/VI

FAILURE OF IMMUNE RESPONSE TO SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* O157 LIPOPOLYSACCHARIDE ANTIGEN IN HEALTHY CARRIERS

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Several studies have confirmed that patients with STEC O157 infection frequently develop IgG, IgM, and IgA classes of antibodies against O157 lipopolysaccharide (LPS) antigen. In 1996, an outbreak of STEC O157 infection arose in an asylum in Japan, involving four healthy carriers. We monitored humoral immune response (IgM) against STEC O157 LPS antigen in patients and healthy carriers in the asylum by using enzyme-linked immunosorbent assay (ELISA). IgM antibodies against STEC O157 LPS were detected in sera of patients. However, 8 days after isolation of STEC O157 from stool samples of healthy carriers, the ELISA values of their sera were negative for IgM antibodies against STEC O157 LPS. We could not detect IgM antibodies against STEC O157 LPS in the sera of these healthy carriers till 23 days after the isolation. It should be noted that care should be taken while diagnosis for STEC O157 infection by immunological test alone.

MOLECULAR CHARACTERIZATION OF THE LARGE PLASMID PO157 AND ITS DISTRIBUTION AMONG ENTEROHEMORRHAGIC *ESCHERICHIA COLI* STRAINS

V165/VI

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We have characterized plasmid pO157 of enterohemorrhagic *E. coli* (EHEC) O157:H7 strain EDL 933 on a molecular level. Following the construction of a physical map, four determinants have been characterized and mapped on this plasmid. A RTX operon could be demonstrated to encode the pore-forming EHEC-hemolysin. Closely to that operon, the *etp* gene cluster presumably encoding a type II secretion system could be identified. In addition, the bifunctional catalase-peroxidase KatP and a novel serine-protease, designated EspP, were shown to be encoded by pO157. These determinants were characteristic for EHEC and did not occur in enteropathogenic, enteroinvasive, enterotoxinogenic or enteroaggregative *E. coli* strains. Hybridization and PCR analyses with probes and primers derived from sequences specific for these genes have shown that plasmids of EHEC strains differ with respect to their gene composition. Detection of plasmid specific genes may be useful for molecular typing of EHEC strains.

MOLECULAR TYPING OF VEROTOXIGENIC *Escherichia coli* USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS

V169/VI

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Amplified fragment length polymorphism (AFLP) was investigated using ABI 377 DNA Sequencer and GeneScan Software for the molecular typing of verotoxigenic *E. coli* (VTEC) strains. The VTEC strains used in this study included serotypes O157:H7 (34 strains), O103:H2 (24), O132:NM (11), O80:NM (3) and O7:H4 (4), which were previously classified by Pulsed-Field Gel Electrophoresis (PFGE) using *Xba*I digestion. Of the 34 O157:H7 strains, 27 were from one Ontario farm (human, water and animal isolates), and showed one basic AFLP banding pattern distinct from those of 7 other strains. Grouping of the O157:H7 isolates by AFLP was the same as determined by PFGE. Among 42 non-O157:H7 VTEC strains (human and animal origin), AFLP patterns differed between serotypes, and within serotypes isolates from the same location had the same unique patterns. Two unrelated strains of O103:H2 and 8 strains of O132:NM were distinguishable by AFLP but not by PFGE. This study demonstrated that AFLP was a useful method for typing of VTEC and was at least as useful as PFGE in discriminating individual isolates.

V170/VI AN AUTOMATED FLUORESCENT PCR METHOD FOR THE DETECTION OF VEROTOXIGENIC *Escherichia coli* ISOLATED FROM ANIMAL, FOOD AND HUMAN SOURCES AND IN SPIKED GROUND BEEF

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An automated fluorescence-based PCR system, AG-9600 AmpliSensor Analyzer, was investigated for the detection of verotoxigenic *Escherichia coli* (VTEC). The AmpliSensor PCR assay involves amplification-mediated disruption of a fluorogenic DNA signal duplex (AmpliSensor) that is homologous to conserved target sequences within a 323-bp amplified fragment of the VT1, VT2 and VTE genes. Using the AmpliSensor assay, 114 strains of VTEC, comprising 49 different serotypes, were detected while 16 strains of non-VT producing *E. coli* and 69 strains of other enteric and food-borne bacteria were not detected. The detection limit of the assay was 1-5 colony forming units (cfu) per PCR reaction using 5 reference VTEC strains. When VTEC cells were added to overnight pre-enriched ground beef samples, the detection limit was 10^3 cfu/mL. The assay was up to two orders of magnitude more sensitive than detection by ethidium bromide-stained agarose gel electrophoresis. The system offers an automated PCR-based detection method for all serotypes of VTEC in food or clinical samples.

V183/VI **DEVELOPMENT OF MONOCLONAL ANTIBODY-BASED SANDWICH E.L.I.S.A'S FOR THE RAPID DETECTION OF VEROTOXIN PRODUCTING *ESCHERICHIA COLI***

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The cell attachment of VTEC strains is thought to involve the 60mDa plasmid coded fimbriae and chromosomal attachment and effacing (eae) coded 94-97kDa outer membrane protein (omp) intimin. Monoclonal antibodies were raised to O26 intimin and used to develop a sandwich ELISA. The assay was used in a survey of enteric animal diseases submitted to a Diagnostic Laboratory for examination.

Detection of Shiga Toxin(Stx)-Producing *Escherichia coli*(STEC) Influenced by Various Culture Conditions

V196/VI

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Currently over 50 serotypes of STEC other than E. coli O157:H7 have been associated with a variety of diseases in humans, including hemorrhagic colitis and hemolytic uremic syndrome. The expression of Stx is the single factor common to all STEC. Therefore it is necessary to detect Stx in a sensitive and specific manner. There are indications that some STEC-isolates cannot be detected by ELISA. Using an ELISA based on hydatid fluid as Stx receptor and monoclonal antibodies (13C4, 11E10) as Stx detector, a number of STEC isolates from humans and cattle was tested for Stx production under various conditions. When cultured in mTSB without shaking some STEC isolates did not produce detectable amounts of Stx. After shaking for 18 h, all isolates had produced Stx detectable by ELISA. In most cases, the production of Stx was further increased by addition of mitomycin C(MMC) especially in cultures from low Stx producers. The results demonstrate that it is possible to detect low Stx producers too after overnight shaker culturing in a broth with MMC.

SERODIAGNOSIS OF *ESCHERICHIA COLI* O157 INFECTION AND ITS USE IN EPIDEMIOLOGICAL STUDIES.

V198/VI

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As part of the investigation by the Laboratory of Enteric Pathogens to determine the incidence of human infection with Vero cytotoxin-producing *Escherichia coli* belonging to serogroup O157, an ELISA based serodiagnostic test was developed. This test specific for antibodies (primarily of the IgM class) specific to *E. coli* O157 was offered for the testing of sera from patients with bloody diarrhoea or haemolytic uraemic syndrome particularly when no bacterial pathogen had been isolated. During the period January 1992 to December 1996, the LEP received 980 sera predominantly from sporadic cases or from small family outbreaks. Of these sera 456 were from patients with HUS and 43% of these cases were confirmed by serodiagnosis alone. There were two outbreaks during this period; outbreak 1 in 1994 and outbreak 2 in 1995 which were identified by the use of serodiagnosis for antibodies to *E. coli* O157 LPS. The use of an ELISA based serological test for the detection of antibody to *E. coli* O157 LPS provides a valuable procedure for the detection of evidence of infection with *E.* coli O157 and an important addition to bacteriological detection procedures.

V211/VI IMPROVED DETECTION AND ISOLATION OF VEROTOXIGENIC *ESCHERICHIA COLI* IN MIXED CULTURES

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Isolation of verotoxin (VT)-producing *Escherichia coli* (VTEC) of any serotype directly from samples or from mixed cultures is laborious, time consuming and often unreliable. A simple and effective method for isolating VTEC was developed by combining growth of liquid samples or toxin-positive mixed cultures on hydrophobic grid membrane filters (HGMF) with immunocapture of VTs on a membrane placed between the HGMF and the agar medium. Removal and immunostaining of the membrane reveals microdots of captured toxins secreted by corresponding VTEC micro-colonies on the overlying HGMF. The VT-positive colonies can be picked directly from the HGMF for further characterization. In conjunction with a sensitive immunoassay for VTs, the membrane technique enables reliable and efficient isolation of any serotype of VTEC from food and fecal samples in 24 to 48 h. Also, the membrane procedure can be modified for quantitation and for concurrent immunodetection of other relevant antigens, such as O157 lipopolysaccharide.

V218/VI **RAPID DISAPPEARANCE OF FECAL VEROTOXINS IN PATIENTS WITH VTEC O157:H7 INFECTION WHO WERE GIVEN ANTIBIOTICS AT EARLY STAGE OF THE DISEASE**

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Fecal verotoxin levels of 22 cases of VTEC O157:H7 ($VT1^+$, $VT2^+$) infection were measured by using Novapath-EHEC (Premier EHEC) kit. Stool samples were applied to the ELISA-based kit which can detect VTs at 5-100 pg/ml range. 20 cases were given antibiotics in early stage whose fecal toxin disappeared in three days. Toxin levels at early stage of the disease were around 100-200 pg/ml , but after 4 to 5 days, VT was not detectable. One patient who did not take any antibiotic was found that on day 7th, the toxin level was still at 50-100 pg/ml range. The specific treatment against toxins, such as an absorbent or anti-verotoxin antibodies should be given within 3 days of the onset.

DETECTION OF EHEC IN MEATS BY DNA PROBES AND ELISA V224/VI

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EHEC infections cause outbreaks of bloody diarrhea and HUS. Bovine meats are considered the principal vehicles of transmission. 35% of cows and 69% of pigs slaughtered in Santiago are colonized by EHEC (Borie C., Prado V., et alt., ICAAC 1996). Rapid diagnostics tools for EHEC are needed to prevent transmission. We evaluated biotinylated DNA probes (SLTI, SLTII, eae and plasmid associated fimbra genes) and an ELISA test (EHEC Premier, Meridian OH) in 67 randomly selected supermarket meat samples. EHEC was detected in 7 (11.1%) by both techniques; in 11 (16.6%) by DNA probes alone, and in 17 (25%) by ELISA alone. 32 samples (47.2%) gave negative results by both techniques. Concordance between DNA probes and ELISA was 58.3% . Most frecuent serogroups found were: O157, O128 and O158.
These results indicated that both techniques might be adecuated as screening tests. EHEC ELISA Premier is easier and faster to do, however positive results should need confirmation by the more specific molecular techniques.

COMPARISON OF GB3 ELISA ASSAY AND PCR FOR DETECTION OF EHEC IN BOVINE AND MEAT SAMPLES. V226/VI

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Verocytotoxin-producing *E.coli* (VTEC) is a known important cause of hemorrhagic colitis and HUS in children in Argentina. VTEC infections have been recognized as a major public health concern worldwide, clearly linked to eat contaminated meat; because of this, a rapid and reliable laboratory method are needed to facilitate the diagnosis. Since no data are available about Gb3 ELISA as a screening test to detect EHEC, the objective of this study was to compare Gb3 ELISA method versus PCR for detection of VTEC strains from meat and bovine samples in Argentina. Four hundred and twenty-three *E.coli* strains were isolated from raw ground beef and hamburgers from different manufacturers and butcher's shops ; and 140 *E.coli* strains were collected from beef cattle (healthy or with diarrhea) at slaughters or farms and dairy calves in farms. Results : 179/563 (31.8%) strains were positive for both methods ; 40/563 (7.1%) of Gb3 ELISA and 58/563 (10.3%) were positive for PCR only. Contingency table analysis of the association between Gb3 ELISA and PCR methods showed a strong correlation (chi square 147.99, $P<0.0001$). Conclusions : Gb3 ELISA assay have showed to be a reliable method and cheaper for screening to detect EHEC in our setting.

V229/VI The development of a PCR specific for *Escherichia coli* 0157.

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A specific polymerase chain reaction (PCR) for detection of *E. coli* 0157 is described. A single pair of oligonucleotide primers were designed to amplify a 479 bp fragment of an *rfb* gene. The primers amplified products of the appropriate size from 39 O157 strains (clinical and animal) but not from non-0157 STEC, closely related *E.* coli O55:H7 and other genera tested. The method was more reliable and easier to read than two O157 agglutination kits. Raw milk was inoculated with a clinical and cattle 0157 isolate and enriched for 24h in mTSB at 37°C. Amplification products were detected when <1 CFU per mL were inoculated into the raw milk. The PCR assay was used to screen 147 raw milk samples from dairy farms in Eastern Australia and no positive samples were detected by PCR or culture. The procedure offers a rapid and sensitive method for screening and typing *E. coli* O157.

V232/VI TOWARD CHARACTERIZING THE H.U.S. PHENOTYPE.

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Enterohemorrhagic *E. coli* (EHEC) strains, most notably *E. coli* O157:H7, are the primary cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS). Pathogenesis is incompletely understood and predicting the onset of this post-diarrheal syndrome is difficult. Both basic research and clinical case management would benefit from determination of specific EHEC features associated with HUS potential. Preliminary *in vitro* studies suggest a correlation between restriction pattern and HUS phenotype in isolates of *E. coli* O157:H7. An interdisciplinary collaborative effort is in progress to: 1) perform RFLP analysis on O157:H7 isolates complete with demographic data; and 2) examine various serotypes of enterohemorrhagic *E. coli*. Our experimental methods are designed to test our hypothesis that there are enterohemorrhagic *E. coli* strains, definable by genotype, which are particularly prone to cause HUS. Expected data should contribute to improved understanding of the pathogenesis of HUS through the combination of biochemical and molecular means of analysis.

A NATIONAL NETWORK FOR MOLECULAR SUBTYPING OF *ESCHERICHIA COLI* O157:H7

V237/VI

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We have begun efforts to establish a national network for subtyping *E. coli* O157:H7 isolates using pulsed-field gel electrophoresis (PFGE). Eight laboratories participated in a study to determine the reproducibility of PFGE in different laboratories using a standarized protocol. Results were highly reproducible when bands smaller than 40kb were excluded from consideration, and strain discrimination was only slightly reduced. A national electronic database of PFGE subtypes is under construction. Four state health department laboratories have direct computer access to the database, which also contains epidemiologic information about each strain. The ability to perform real-time comparisons with patterns in a national database should facilitate recognition of diffuse outbreaks. Additional laboratories will be added in the next year, and the database will be expanded to include other foodborne pathogens.

THE MASSIVE OUTBREAK OF ENTEROHEMORRAGIC E.COLI O-157 INFECTIONS BY FOODS POISONING AMONG THE ELEMENTARY SCHOOL CHILDREN IN SAKAI,JAPAN IN 1996

V6/VII

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In the middle of July in 1996, massive outbreak of hemorrhagic colitis(HC) occurred among the elementary school children in Sakai city. This is the most wide spread outbreak of O-157 infection ever experienced to our knowledge. Most patients developed typical symptoms of HC with severe abdominal pain and bloody diarrhea. Enterohemorrhagic E.coli O-157:H7 producing both VT1 and VT2 was detected in stool samples from a half of the victims. Almost definitely, lunch foods supplied for 32551 children in 62 elementary schools in Sakai were contaminated by E.coli O-157. 6309 children suffered from HC. Among them,102 children developed hemolytic uremic syndrome(HUS) after HC and three girls have died.

V8/VII

IN VITRO ADHESION OF A VEROCYTOTOXIN PRODUCING *ESCHERICHIA COLI* IS REDUCED BY COINCUBATION WITH A *LACTOBACILLUS* SPECIES

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Biotherapeutic agents may offer an alternative to antimicrobials and have the property of an immediate onset of action. As epithelial cell adhesion is important in the pathogenesis of disease caused by enterohemorrhagic *E coli* (EHEC), we examined whether adhesion of EHEC strain CL-8 (serotype O157:H7), known to exhibit attaching and effacing adhesion both in vivo and in vitro, was altered in the presence of *Lactobacillus plantarum* strain 299v. Both strains were grown overnight at 37°C, then collected and reconstituted in PBS (pH7.4) prior to addition to cell growth media of HT29 cells. Binding of 1 x 10^5 CL-8 per well with increasing amounts of *L plantarum* was quantified by CFU determinations. Coincubation of *L plantarum* with CL-8 showed quantitative inhibition with $1x10^9$ *L plantarum* reducing binding by 98% compared to control ($1.6x10^4$ CFU±$0.9x10^4$ CFU vs $2.93x10^2$ CFU±$0.3x10^2$ CFU, $p<0.001$). We conclude that a non-infectious constituent of the intestinal microbiota is capable of reducing EHEC epithelial cell adhesion in vitro.

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TREATMENT OF HUS AND ITS COMPLICATIONS

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We present data on the outcome of 65 patients with D+ HUS referred to CHOP from 1987 to 1996. We evaluate critically each statement we make in regard to management. Improvements in treatment include judicious use of blood transfusions, careful control of blood pressure, appropriate use of dialysis and intravenous alimentation, avoidance of unproved measures, and avoidance of anti-coagulants, plasmapheresis and platelet infusions. We restrict the use of invasive vascular monitoring. To minimize risks renal biopsies are not done and dialysis is discontinued as soon as possible. We eschew the view that anuric renal failure can be prevented by giving fluid challenges and furosemide infusions. With the advent of CVVHD, however, we give fluids to hypovolemic patients with hypotension without fear of fluid overload. An important factor in outcome is early colectomy for intestinal gangrene. A single patient has died within 12 hours of admission.

HUMANIZATION OF MONOCLONAL ANTIBODIES AGAINST *ESCHERICHIA COLI* TOXINS STX1 AND STX2

V110/VII

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The murine monoclonal antibodies 13C4 and 11E10 are specific for the Shiga toxins types 1 and 2, respectively, that are expressed by Enterohemorrhagic *E. coli*. These antibodies are capable of neutralizing the toxins both in tissue culture and animal models. For the purpose of developing therapeutic agents to treat or prevent hemolytic uremic syndrome, we have humanized these monoclonals. Total RNA from the hybridoma cell lines and mouse antibody variable region primer sets were used for RT-PCR to amplify the variable regions. The V regions were then cloned into a mammalian expression vector for the production of mouse variable region:human IgG1/kappa chimeric antibodies. NS0 cells were transfected with the vector and the humanized antibodies produced recognize the toxins in an enzyme immunoassay. The protective capacity of these antibodies in an animal model system is being tested and the results will be discussed.

THE RESISTIVE INDEX IN D+ AND D- HUS: IS THERE A CLINICAL CORRELATION?

V111/VII

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Several reports have documented the utility of the resistive index (RI) obtained with Doppler sonography in the acute phase of HUS as clinically significant and a potential guide to therapy. We analyzed our experience with RI in children with both D+ and D- HUS with a view toward predicting the need for therapy and prognosis. Sixteen children with HUS had renal Doppler ultrasonography early in the course of their illness. Eleven children, mean age 7.0 y had D+ HUS, the remaining 5, mean age 0.9 y had D- HUS [Denys-Drash (2), meningococcemia, *S. pneumoniae* and idiopathic]. RIs were determined blindly without knowledge of the type of HUS and read as normal or elevated for age. Abnormal RIs were observed in 6/11 children with D+ HUS. Anuria was present in only 3/6 cases, all have normal renal function on follow-up. Of the 5 with normal RIs, 3 had anuria, 1 has decreased renal function. All 5 patients with D- HUS had normal RIs; 4 required dialysis. 2 have normal renal function. We conclude that the RI offers no value in determining the need for dialysis and should not be performed routinely. Patients with D- HUS who would be expected to have increased renovascular resistance by the nature of their pathology did not demonstrate this abnormality on Doppler sonography.

V149/VII THERAPEUTIC VALUE OF STX-SPECIFIC ANTIBODIES OR SYNSORB IN STREPTOMYCIN (STR)-TREATED MICE ORALLY INFECTED WITH SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC)

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The purpose of this study was to assess the protective efficacy of anti-Stx2 antibodies or the chemiabsorbant Synsorb against death of str-treated mice fed virulent Stx2- or Stx2-variant producing STEC. Monoclonal antibody (Mab against Stx2 A subunit) 11E10 administered intraperitoneally 1 day before and at infection completely protected 5/5 CD-1 mice challenged with Stx2-variant producing strain B2F1 and also provided significant protection to DBA/2J mice against challenge with Stx2-producing strain 86-24 (2/3 survivors). Pretreatment with Mab 13C4 (against Stx1 B subunit) was not protective against either challenge strain. Str-treated CD-1 mice that were fed Synsorb before or directly after challenge with B2F1 did not display increased rates of survival compared to animals fed Chromosorb but did show an average increase in mean time to death (MTD) of 1 day. Although passive immunization with type specific antibodies was more efficacious in this model than Synsorb, we are testing the possibility that antibodies can be adminstered later in the infection if mice are also given Synsorb.

V157/VII **HUMAN MILK LIPIDS BIND SLT-I**

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HUS is rare before 6 months of age. Immunologic and non-immunologic factors present in breast milk may partially explain this observation. In prior studies we have demonstrated that human milk contains Gb_3, the receptor for the B subunit of Shigatoxin (ST) and Shiga-like toxins (SLTs). We therefore sought to determine if the lipid component of breast milk binds purified SLT-I. Breast milk samples obtained from healthy donors were centrifuged at 14,000 xg for 30 minutes, the soluble fraction discarded, and the lipid layer collected. An emulsion of equal volumes of the lipid layer of each sample and purified SLT-I [3.7×10^{-8} M] was incubated at 37 °C in a rotatory shaker. The lipid layer with bound toxin was separated by centrifugation. The amount of free SLT-I present in the aqueous fraction was determined by Gb_3 ELISA. The lipid layer bound an average of 95.7% of SLT-I (range 92.8%-99.9% for milk lipid extracts from various women). These results are consistent with the hypothesis that toxin binding lipids present in human milk are biologically active and may contribute to the putative protective effect.

SURVEY OF IN VITRO SUSCEPTIBILITIES TO ANTIMICROBIAL AGENTS OF ENTEROHEMORRHAGIC ESCHERICHIA COLI ISOLATED FROM JAPAN IN 1996

V172/VII

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Enterohemorrhagic Escherichia coli (EHEC) colonizes human intestines and causes watery diarrhea and hemorrhagic colitis, followed by development of hemolytic uremic syndrome due to Vero toxins. It is presumed that the initial infections can be treated by chemotherapy with antimicrobial agents. In Japan, large explosive endemics due to serotype O157:H7 strains occurred in 1996. In this study, we investigated in vitro susceptibilities to 42 antimicrobial agents of EHEC strains (192 strains) that were isolated from 18 prefectures in Japan in 1996. Suceptibility testing was done by the agar dilution method. The lowest MIC values for fosfomycin was obtained when serum or blood was added to the media (MIC90: 2 mg/ml). The MIC90 values for azithromycin, kanamycin, tetracycline, minocycline, cefditoren/cefteram, and norfloxacin were 8, 2, 2, 2, 0.25, and 0.13 mg/ml, respectively. Drug resistance was observed with fosfomycin, kanamycin, tetracycline, and some others. The susceptibility patterns often varied by isolation place or EHEC serotypes.

CENTRAL SCOTLAND *ESCHERICHIA COLI* 0157 OUTBREAK- (CLINICAL ASPECTS)

V212/VII

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During the central Scotland outbreak of food borne *E coli* 0157 infection 113 cases (27.6% of total outbreak cases) were admitted to the Lanarkshire Area Infectious Diseases Unit. Eighty-six (76.1%) cases had laboratory evidence of infection. Over 50% were over 58 years of age with a female preponderance. All patients by definition suffered diarrhoea, 86.7% also reported abdominal pain, 46.9% vomiting and 42.3 % fever. Blood in stool (92%) correlated with complication development. The overall complication rate was 28.3%, Micro-angiopathic haemolysis (MAH) being the most common complication. Eleven (52.4%)children (under 16 years) and 38 (41.3%) adults developed MAH. Four (36.4%) children and 4(10.5%) adults developed renal failure requiring haemodialysis (HD). Sixteen (42.1%) adults, including all 4 who required HD, were treated intensively with therapeutic plasma exchange and prostacycline infusions. The mortality rate was higher in those receiving plasma exchange (31.2%) and patients with MAH (20.4%) compared with the rate for the total in-patient cohort (9.7%).

During a large outbreak of *E coli* 0157 infection the elderly (> 60 years), in particular older males (>70 years), and children < 5 years appeared most likely to develop a complicated illness and/or death. Other clinical and laboratory predictors of outcome will be discussed.

V219/VII **QUESTIONNAIRE-BASED CLINICAL ASPECTS OF VTEC INFECTION IN JAPAN, 1996**

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We have sent questionnaires to 3908 hospitals all over Japan, and collected the information from 1769 hospitals (45.3%) on VTEC infection in 1996. Culture-confirmed, serologically positive and outbreak-involved 1012 cases were analyzed. Of them 197 (19.5%) developed HUS though less than 2% of HUS was reported from several outbreaks in school children. Complications of neurological manifestation in 49, appendicitis in 27, jaundice in 13, colon invagination in 10, rectal prolapse in 3, and pancreatitis in 3 were reported. Antibiotics treatment within 3 days reduced the risk of HUS three times and caused 1.7 times more rapid recovery than non-antibiotics group.

V220/VII INTENSIVE THERAPEUTIC PLASMA EXCHANGE (TPE) IN AN ELDERLY COHORT OF PATIENTS WITH *E COLI* 0157 RELATED DISEASE

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During the Lanarkshire outbreak of food borne *E coli* 0157 infection over 400 people were infected and many elderly patients required hospital care. The anticipated mortality in those developing Haemolytic Uraemic Syndrome or Thrombotic thrombocytopenic purpura (HUS/TTP) was 20% prompting early intervention with intensive TPE and intravenous prostacycline.

Criteria for TPE were: any degree of red cell fragmentation plus platelet count $<150x10^9/l$ plus LDH>1.5 x upper normal and/or clinical suspicion of HUS/TTP. Prostacycline was given by continuous infusion from first TPE.

18 patients developed HUS/TTP and 16 (median age 71 years: 4 M, 12F) received a total of 80 TPEs. In most prostacycline was discontinued prematurely because of intolerance. Five (31%) died from disease related complications including 2/4 requiring haemodialysis. Two suffered myocardial infarcts; 5 developed severe agitation and/or impaired conscious level. Two developed acute peritonism; 1 haemorrhagic colitis. Complications of TPE included respiratory arrest, extravasation of exchange fluid, hypocalcaemia, hypomagnesaemia and hypogammaglobulinaemia. Rapid onset pulmonary oedema occurred, despite hypovolaemic exchange, in 50% of patients.

Prostacycline was poorly tolerated but TPE was used with apparent

PREVENTION OF ENTEROHEMORRHAGIC COLITIS IN YOUNG RABBITS BY ORAL ADMINISTRATION OF IRRADIATED ESCHERICHIA COLI VACCINE

V43/VIII

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We have searched for an effective oral vaccine which elicits anti-enterohemorrhagic immunity. Groups of young rabbits were orally immunized with three doses of irradiated (by electron high energy) E.coli 0157:H7 vaccine. The vaccine is safe, and ensured marked immunity as shown by development of mucosal (sIgA), humoral (IgG) and cell-mediated immunity (assayed by uptake of ^{3}H-TdR by Payers patches lymphoid cells) as well as by bacteriological, histopatological and electron microscopy findings.

Summing up, these results showed that three oral doses of irradiated E.coli 0157:H7 vaccine provided strong protection against infection with enterohemorrhagic Escherichia coli strains.

BOVINE IMMUNE RESPONSE TO ESCHERICHIA COLI O157:H7

V67/VIII

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We are exploring the bovine immune response to Escherichia coli O157:H7 in order to develop vaccination strategies to reduce fecal shedding of this pathogen in cattle. Three groups of calves were studied. One group was orally vaccinated with an E. coli O157:H7 variant that does not express Shiga-like toxins (SLT) I or II, a second group with wild-type (wt) E. coli O157:H7 (SLT II^{+}), and a third group with a non-pathogenic strain of E. coli. All calves were subsequently challenged with wt O157:H7 three weeks after a second vaccination. Prior exposure to wt O157:H7 or the SLT^{-} variant did not reduce fecal shedding of wt O157:H7 upon challenge. All calves developed antibodies to O-antigen. Peripheral blood mononuclear cells (PBMCs) isolated from the calves vaccinated with the SLT^{-} strain proliferated in response to heat-killed O157:H7, while PBMCs from calves vaccinated with wt O157:H7 or control strain did not. These findings support the hypothesis that SLT II has an immunosuppressive effect which future vaccination efforts will need to address.

V119/VIII **Immunization of Pigs with Verotoxin 2e toxoid and a VT2e B subunit mutant.**

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We studied the immune response and protective efficacy of edema disease toxin (VT2e) toxoid and the B subunit of a VT2e mutant designated GT3 which binds principally to Gb3. Expression systems were developed for both VT2e and GT3 B subunit which yielded 15 and 50 mg per 3 liter culture respectively. GT3 B has a pentameric structure and reacts well with the VT2 B subunit specific MAb BC5BB12.
Pigs immunized with VT2e toxoid developed high titre antibody responses (1:12,800) and were completely protected against intravenous VT2e challenge. Pigs immunized with GT3B failed to develop measurable antibody titres but 5/6 survived the challenge with VT2e without becoming ill. GT3B immunization elicited high titres of anti VT2e antibodies (1:3,200) in rabbits but elicited no antibody response in pigs and mice. GT3B can be produced in large amounts but has little immunogenicity in pigs. We are currently investigating the basis for this.

V143/VIII PROTECTIVE IMMUNITY TO SHIGA TOXIN (STX) 1 FOLLOWING ORAL IMMUNIZATION WITH STX 1 B-SUBUNIT-PRODUCING *V. CHOLERAE* CVD 111

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We have previously shown that the Stx 1B expressing classical cholera vaccine strain CVD 103-HgR(pDA60) induces a systemic and possibly locally protective immune response in rabbits. We have now studied the immune response to an Stx 1B expressing El Tor*V. cholerae* vaccine strain [(CVD 111(pDA60)]. 5 New Zealand rabbits were given CVD 111(pDA60) and 5 given CVD 111 as controls (3 doses, ~10^{10} organism/dose). All experimental rabbits developed high anti-Stx 1B IgG titres. Experimental rabbits secreted significantly less fluid than controls when challenged with Stx 1 in ileal loop experiments (0.38 ml/cm vs 1.12 ml/cm, p=0.04). Stx 1B based oral vaccines can induce local and systemic immune responses.

EXPRESSION OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* INTIMIN IN TRANSGENIC PLANTS: AN EDIBLE ANTI-EHEC O157:H7 VACCINE CANDIDATE

V234/VIII

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Our goal is to produce an edible vaccine that will protect cattle or humans against colonization with Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 and other intimin-expressing *E. coli*. As a first step to achieve this purpose, we have constructed transgenic tobacco plants that express the intimin adhesin. Two intimin-encoding shuttle plasmids were designed, and each was introduced into tobacco by *Agrobacterium*-mediated transformation. Both plasmids expressed Histidine-intimin from a double-enhanced cauliflower mosaic virus 35S-driven promoter. One of the plasmids also contained tobacco nuclear matrix attachment regions to increase transgene expression. Over 100 independent transformant lines were recovered. These tobacco plants were morphologically normal, fertile, and expressed intimin (as estimated by Western blot) at 0 to 0.1% of total plant-associated protein. Thus, we have demonstrated that intimin can be synthesized in plants. Our next step will be to express intimin in canola, a candidate anti-EHEC edible transmission vaccine for cattle.

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